MARINE-DERIVED NUTRIENTS IN RIVERINE ECOSYSTEMS: DEVELOPING TOOLS FOR TRACKING MOVEMENT AND ASSESSING EFFECTS IN FOOD WEBS ON THE KENAI PENINSULA, ALASKA

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A

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Abstract

Marine-derived nutrients (MDN) delivered by spawning Pacific salmon (*Oncorhynchus* spp.) contribute to the productivity of riverine ecosystems. Optimizing methods for measuring MDN assimilation in food webs will foster the development of ecologically based resource management approaches. This dissertation aims to better understand relationships among spawning salmon abundance, biochemical measures of MDN assimilation, and the fitness of stream-dwelling fishes. The goals of my first research chapter were (1) to understand the factors that influence stable isotope ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) and fatty acid measures of MDN assimilation in stream and riparian biota, and (2) to examine the ability of these measures to differentiate among sites that vary in spawning salmon biomass. For all biota studied, stable isotopes and fatty acids indicated that MDN assimilation increased with spawner abundance. Among Dolly Varden (*Salvelinus malma*), larger individuals assimilated proportionately more MDN. Seasonal effects were detected for aquatic macroinvertebrates and riparian horsetail (*Equisetum fluviatile*), but not for Dolly Varden. Of all dependent variables, Dolly Varden $\delta^{15}N$ had the clearest relationship with spawner abundance, making this a good measure for monitoring MDN assimilation. Expanding on these results, two chapters examined potential fisheries management applications. The first sought to identify spawner levels above which stream-dwelling Dolly Varden and coho salmon (*O. kisutch*) parr cease to gain physiological benefits associated with MDN. RNA-DNA ratios (an index of recent growth rate) and energy density indicated saturation responses where values increased rapidly with spawner abundance up to approximately 1 kg/m^2 and then leveled off. In

coho salmon parr, energy density and RNA-DNA ratios correlated significantly with $\delta^{15}N$. These results show strong linkages between MDN and fish fitness responses, while the saturation points may indicate spawner densities that balance salmon harvest with the ecological benefits of MDN. The second application tested a quick and inexpensive method for estimating spawning salmon abundance based on $\delta^{15}N$ in stream-dwelling fishes. Estimates made with coho salmon parr were unbiased, tightly correlated with observed values, and had a mean absolute deviation of 1.4 MT spawner biomass/km. Application of this method would allow estimates of annual escapement to be made on a potentially large number of streams.

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Chapter 1

Introduction

Ecological outcomes of marine-derived nutrient subsidies

Pacific salmon (*Oncorhynchus spp.*) are the dominant predator in the North Pacific Ocean and return annually by the millions to natal streams in North America and Asia. During spawning runs, salmon deliver marine-derived nutrients (MDN; nitrogen, phosphorus, carbon, and other nutrients) in the form of eggs, excreta, and carcasses (Kline et al. 1993, Gende et al. 2004). Salmon carcasses contain nitrogen, phosphorus, and carbon in the same relative proportions as needed for growth by rearing salmon, making MDN an ideal fertilizer for salmon ecosystems (Wipfli et al. 2004). Given the high densities of spawning salmon in some streams, MDN subsidies can be large. Bear Creek, a small southeast Alaska stream used by chum salmon, received 5.3 g P, 37 g N, and 5455 kJ of energy per m^2 of streambed during one spawning run (Gende et al. 2004). In high latitudes, the importance of marine nutrients is magnified by the low ambient nutrient levels in freshwater systems (Gross et al. 1988, Perrin and Richardson 1997). As such, estimates of MDN inputs are most informative when contrasted with estimates of watershed nutrient inputs. In oligotrophic Iliamna Lake, nitrogen inputs from salmon greatly exceed inputs from the watershed and peak sharply with cyclically abundant sockeye runs (Kline et al. 1993).

Where salmon returns remain abundant, MDN influences multiple trophic levels in stream ecosystems. Increased standing stocks of biofilm (Perrin and Richardson 1997,

Wipfli et al. 1998, 1999; Johnston et al. 2004, Mitchell and Lamberti 2005) and macroinvertebrates (Wipfli et al. 1998, 1999, Claeson et al. 2006, Lessard and Merritt 2006, Walter et al. 2006) have been linked to MDN inputs. Stream-dwelling fishes can benefit from MDN subsidies indirectly through increased macroinvertebrate production and directly through the consumption of energy-rich salmon flesh and eggs (Bilby et al. 1996). As a result, growth rates (Bilby et al. 1996, Wipfli et al. 2003, Giannico and Hinch 2007), body condition (Bilby et al. 1998), and energy storage (Heintz et al. 2004) of stream-resident fishes are increased by the availability of MDN, likely leading to increased survival for juvenile salmon (Gardiner and Geddes 1980, Wipfli et al. 2003, Heintz et al. 2004). Riparian soils are fertilized by MDN (Bartz and Naiman 2005, Drake et al. 2005), and associated vegetation can derive a substantial proportion of nitrogen from this pool (Bilby et al. 2003, Reimchen et al. 2003), potentially enhancing growth rates (Helfield and Naiman 2001, 2002, 2003; but see Kirchhoff 2003). Marine derived nutrients may ultimately initiate a positive feedback loop whereby spawning salmon enhance the carrying capacity of spawning systems (Kline et al. 1993, Wipfli et al. 1998, Helfield and Naiman 2001, 2002; Drake et al. 2005).

The ecological importance of MDN can clearly be demonstrated by cases where MDN supplies are disrupted by salmon population crashes. The prolonged depression of salmon stocks in the Columbia River basin due to dams and habitat loss is a prime example. Here, a chronic nutrient deficiency hinders the recovery of endangered and threatened Pacific salmon stocks (Gresh et al. 2000, Petrosky et al. 2001, Achord et al. 2003, Peery et al. 2003, Scheuerell et al. 2005, Zabel et al. 2006) and diminishes the

potential of expensive habitat improvement projects (Gresh et al. 2000). Density-dependent mortality has been documented among juvenile chinook, despite the fact that populations have been reduced to a fraction of historic levels, suggesting that nutrient deficits have reduced the carrying capacity of the Columbia River basin (Achord et al. 2003, Scheuerell et al. 2005). A population viability analysis has indicated that declines in MDN have very likely contributed to low productivity of juvenile salmon and that increasing the productivity could lead to large increases in the salmon population (Zabel et al. 2006). This situation presents a negative feedback loop where the decline in spawner abundance diminishes the capacity of streams to produce new spawners (Levy 1997). Fisheries managers recognize this and are incorporating strategies to restore freshwater nutrient stores into salmon recovery programs (Stockner 2003).

Evolutionary basis for marine-derived nutrient subsidies

Given the positive effects of MDN on stream-dwelling salmonids and their environment, it is tempting to infer that Pacific salmon return to their natal streams, spawn, and die in order to create a fertile rearing environment for their progeny. While rearing environments are certainly enhanced by spawning salmon, the life history adaptations that lead to this outcome – anadromy, homing, and semelparity – have evolved for reasons altogether unrelated. Fertilization of spawning environments by Pacific salmon, therefore, appears to be a fortuitous by-product of their life cycle.

Anadromy is an important step in the fertilization process because it creates the opportunity for salmon populations to assimilate large quantities of allochthonous carbon

and nutrients. The food availability hypothesis (sensu Maekawa and Nakano 2002) predicts that diadromy evolves in instances where a disparity in productivity exists between adjacent freshwater and marine environments (Gross et al. 1988). Freshwater primary productivity generally declines with latitude, and freshwater productivity in the spawning range of Pacific salmon is half (or less) of that in lower latitudes (Gross et al. 1988). Conversely, ocean primary productivity generally increases with latitude, peaking within the range of Pacific salmon (50 – 55° N; Gross et al. 1988). As such, the evolution of anadromy in Pacific salmon is thought to be a response to the reproductive fitness gained by exploiting the relatively rich marine environment. For Pacific salmon, the ocean phase growth rate of anadromous forms is typically far greater than that of conspecifics remaining in freshwater (McDowall 2001).

Semelparity is a life history trait by which organisms maximize the investment in one reproductive effort at the expense of any future reproductive effort. In terms of MDN subsidies in fresh waters, semelparity is synergistic with anadromy in that, through the post-spawning death of Pacific salmon, MDN becomes biologically available to stream and riparian ecosystems. In salmon, it may have evolved as a response to the high cost of migration to natal streams and the associated reduction in adult survival (Roff 1988). Crespi and Teo's (2002) phylogenetic analysis showed that the evolution of semelparity in Pacific salmon was accompanied by increased egg size. They noted that, while long migrations may have been a prerequisite, the driving force behind the evolution of semelparity was the afforded increase in egg mass and associated increase in

juvenile survival. Their work also supported the life history hypothesis that fewer reproductive events correspond to increased effort per event.

Homing, whereby reproductive adults return precisely to the same environment in which they were spawned, is an iconic trait of Pacific salmon. The propensity for salmon to range widely throughout their life cycle necessitates a mechanism by which salmon return to suitable spawning sites. By this means, salmon increase juvenile survival by returning to spawn in an environment whose suitability is self evident (Cury 1994). Another adaptive advantage of homing is that it enables the evolution of unique stocks that are adapted to local conditions (Dittman and Quinn 1996). Without homing, gene flow would occur throughout the species and adaptation to specific freshwater conditions would be impossible. In this sense, homing counteracts the dispersal effects of anadromy (McDowall 2001). In terms of MDN subsidies, homing concentrates MDN effects in those areas where salmon spawn, potentially improving habitat conditions for subsequent generations of conspecifics. For example, increased tree growth rates associated with MDN inputs may lead to higher quality spawning and rearing habitat (Helfield and Naiman 2001). For salmon species with extended fresh water rearing stages, homing ensures that salmon eggs, flesh, and nutrients will be deposited in proximity to juvenile conspecifics.

Although anadromy, homing, and semelparity evolved in response to different selective pressures, they collaborate to subsidize rearing streams with MDN. Considering the evidence suggesting a positive feedback mechanism whereby spawning salmon increase the growth rate (and, by extension, survival) of their progeny, it stands to

reason that the life history traits contributing to MDN fluxes are in part maintained by the reproductive advantages offered by MDN subsidies.

Tracking marine-derived nutrient subsidies in food webs

The assimilation of MDN into freshwater food webs can be tracked biochemically, and these tracking methods form the basis for potential MDN-based fisheries management approaches. Approaches using naturally occurring stable isotopes of nitrogen and carbon (expressed as $\delta^{15}N$ and $\delta^{13}C$, respectively) have been used extensively for this purpose. The isotopic composition of adult Pacific salmon, where most individuals range between 10 and 15‰ for $\delta^{15}N$ and between -21 and -19‰ for $\delta^{13}C$ (Welch and Parsons 1993, Satterfield and Finney 2002), is enriched relative to freshwater sources (e.g., Kline et al. 1990). The isotopic disparities between these nutrient pools offer a means for estimating the subsidy of MDN to freshwater food webs (Kline et al. 1990, Bilby et al. 1996, Chaloner et al. 2002, Hicks et al. 2005, Scheuerell et al. 2007). For example, Bilby et al. (1996) found that juvenile coho salmon (*O. kisutch*) in Washington spawning streams derived 30% of their nitrogen and 37% of their carbon from spawning salmon.

Fatty acid analysis is another method for understanding food web relationships that can be used to track MDN assimilation in stream food webs. Ingested fatty acids, unless directly catabolized for energy, are stored in lipid reserves with little or no modification. As such, the fatty acid composition of a consumer reflects that of its food sources, with individual fatty acids occurring in proportion to that found in the combined

food items (Iverson et al. 2004). As with stable isotopes, the relative contribution of multiple food sources to a consumer is best resolved when the food sources have disparate signatures. Production of different polyunsaturated fatty acids by freshwater (including riparian) and marine producers leads to distinct fatty acid signatures for marine and freshwater consumers (Ackman 1999, Napolitano 1999). This disparity is most evident in the relatively high content of ω3 fatty acids relative to ω6 fatty acids in marine fishes; ω3:ω6 ranges from 0.5 to 3.8 in freshwater fish and from 4.7 to 14.4 in marine fish (Henderson and Tocher 1987). The fatty acid signatures of freshwater fish that assimilate MDN come to reflect the marine source, allowing for inferences regarding the contribution of MDN to consumer diets (Heintz et al. 2004, Volk 2004). One main drawback to the use of fatty acids is that the per-sample cost for laboratory analysis is substantially greater than that for stable isotope analyses.

Marine-derived nutrients, fisheries management, and the goals of this thesis

Although the effects of MDN subsidies on freshwater ecosystems have been documented, a better understanding of the strengths and limitations of different approaches to tracking MDN assimilation is needed. In particular, knowing the extent to which these approaches are influenced by non-MDN variables would facilitate long-term and spatially extensive monitoring of MDN assimilation, thereby providing a more detailed understanding of the effects of fluctuating salmon abundance and other ecological conditions on natal stream ecosystems and associated food webs. Optimizing methods for measuring MDN assimilation in stream-riparian food webs and

understanding the temporal and spatial variation in MDN assimilation will foster the development of ecologically-based watershed and fisheries management approaches. As such, the goals of my first chapter were (1) to understand the factors that influence various stable isotope ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) and fatty acid ($\omega3$:$\omega6$ and a multivariate distance metric) measures of MDN assimilation in stream and riparian biota and (2) to examine the ability of these measures to differentiate among sites that vary in spawning salmon biomass.

Expanding on the results presented in the first chapter, two subsequent chapters examined potential fisheries management applications that capitalize on quantitative relationships among MDN abundance, assimilation measures, and effects on stream-dwelling fishes. Incorporating MDN budgets into fisheries and ecosystem management strategies requires a better understanding of the dose-response relationship between salmon escapement and the ecological benefits of MDN (Wipfli et al. 1999, Bilby et al. 2001, Gende et al. 2002, Wipfli et al. 2003). Toward that end, Bilby et al. (2001) reported an asymptotic relationship between coho salmon parr $\delta^{15}N$ and spawner abundance, which they proposed as a method for identifying MDN assimilation capacity and, in turn, for setting salmon escapement goals that provide adequate MDN for stream-dwelling fishes. While the overall approach is intuitively appealing, some problems may arise from the use of stable isotopes in this context. Stable isotopes confer no direct information about fish fitness and, further, can be confounded by factors both internal and external to the fish. Since growth and energy storage in stream-dwelling fish have been linked to MDN abundance (Wipfli et al. 2003, Heintz et al. 2004), a more

appropriate and direct measure of a fish population's capacity to utilize MDN may be the spawner levels above which stream-dwelling fish cease to gain these physiological benefits. As such, the goal of the first management application was to examine the relationship between salmon spawner abundance and growth rates and energy density in stream-dwelling Dolly Varden (*Salvelinus malma*) and coho salmon parr. These results will show the strength of linkages between MDN and fitness responses and determine whether saturation responses in fitness measures can indicate spawner densities that balance salmon harvest with the ecological benefits of MDN in stream-dwelling fishes.

The second management approach also capitalizes on the quantitative relationship between spawner abundance and MDN effects. Here, the goal was to develop and test models that estimate spawner abundance at a given stream reach based on the nitrogen isotopic composition of stream-dwelling fishes captured there. The potential of such an approach has been indicated recently (Reichert et al. 2008), yet the method has not been adequately developed or tested. This method has the potential to substantially increase the number of individual salmon stocks that can be monitored annually since the required field effort is minimal and the analytical expenses are low. Refinement and widespread application of an isotope-based escapement index would also facilitate long-term and spatially extensive monitoring of MDN assimilation as a means to understand the potential effects of fluctuating salmon abundance and other ecological conditions on the food webs of salmon streams.

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Chapter 2

The effect of spawning salmon abundance on the stable isotope ratios and fatty acid composition of stream and riparian biota[1]

ABSTRACT

Marine-derived nutrients (MDN) delivered to streams by spawning Pacific salmon are an ecologically important resource flux that contributes to the productivity of riverine ecosystems. Optimizing methods for measuring MDN assimilation in stream-riparian food webs and understanding the temporal and spatial variation in MDN assimilation will foster the development of ecologically based watershed management approaches. Our main objectives were (1) to understand the factors that influence various stable isotope and fatty acid measures of MDN assimilation in stream and riparian biota and (2) to examine the ability of these measures to differentiate among locations that vary in spawning salmon biomass. We sampled biota spring through fall in each of three regions on the Kenai Peninsula that differ in climate, geology, and, in turn, ambient nutrient concentrations. Within each region we sampled three streams (two with salmon and one without) along a three-station gradient from headwaters to river mouth. We used Akaike Information Criterion (AIC_c) model selection to develop multiple linear regression models expressing carbon, nitrogen, and sulfur stable isotope ratios and fatty acids (ω3:ω6 and multivariate distance from salmon flesh) as a function of spawning

[1] Rinella, D.J., M.S. Wipfli, C.A. Stricker, and C. Walker. The effect of spawning salmon abundance on the stable isotope ratios and fatty acid composition of stream and riparian biota. Prepared for submission to Transactions of the American Fisheries Society.

salmon abundance and selected covariates. Candidate models expressed spawning salmon abundance in terms of either local spawner density (LSD) or cumulative upstream spawner biomass (USB) to determine which parameter was the better predictor. LSD, region, and Dolly Varden (*Salvelinus malma*) length were the best predictors of MDN assimilation in Dolly Varden. LSD or USB plus season and region were the best predictors of MDN assimilation in macroinvertebrates and horsetail (*Equisetum fluviatile*). While confirming the importance of spawner abundance, these results indicate that MDN signals are maintained year-round in Dolly Varden but not in macroinvertebrates and horsetail, that inference must be made on a region-by-region basis, and that larger Dolly Varden assimilate proportionately more MDN. Of all dependent variables, Dolly Varden $\delta^{15}N$ and $\delta^{34}S$ had the most precise relationship with spawner abundance and the greatest effect size, making these the best measures for monitoring MDN assimilation in stream food webs. When the effects of region and fish length are accounted for, Dolly Varden $\delta^{15}N$ and $\delta^{34}S$ can be used to estimate the abundance of salmon spawning in stream reaches. These results show that both fatty acids and stable isotopes are reliable indicators of salmon spawner presence and abundance in streams, and both show promise as tools for monitoring anadromous fish densities and MDN levels in watersheds. Stable isotopes may be more practical because they are easier to sample and handle, cheaper to process and analyze, and because data are easier to interpret.

INTRODUCTION

Marine derived nutrients (MDN), conveyed inland by spawning Pacific salmon (*Oncorhynchus* spp.) in the form of eggs, excreta, and carcasses, are recognized as an ecologically important flux of energy and nutrients to stream and riparian ecosystems (Kline et al. 1997, Levy 1997, Willson et al. 1998, Gende et al. 2002, Naiman et al. 2002, Schindler et al. 2003). Where salmon returns remain abundant, MDN influences multiple trophic levels in stream ecosystems. Increased standing stocks of biofilm (Wipfli et al. 1998, 1999; Johnston et al. 2004, Mitchell and Lamberti 2005) and macroinvertebrates (Wipfli et al. 1998, 1999, Claeson et al. 2006, Lessard and Merritt 2006, Walter et al. 2006) have been linked to MDN inputs. Growth rates (Bilby et al. 1996, Wipfli et al. 2003, Giannico and Hinch 2007), body condition (Bilby et al. 1998), and energy storage (Heintz et al. 2004) of stream-resident fishes are increased by the availability of MDN, likely leading to increased survival for juvenile salmon (Gardiner and Geddes 1980, Wipfli et al. 2003, Heintz et al. 2004). Riparian soils are enriched by MDN (Bartz and Naiman 2005, Drake et al. 2005), and associated vegetation can derive a substantial proportion of nitrogen from this pool (Bilby et al. 2003, Reimchen et al. 2003), potentially enhancing growth rates (Helfield and Naiman 2001, 2002, 2003; but see Kirchhoff 2003). Marine derived nutrients may ultimately initiate a positive feedback loop whereby spawning salmon enhance the carrying capacity of natal streams (Wipfli et al. 1998, Helfield and Naiman 2001, 2002; Drake et al. 2005).

Approaches using naturally occurring stable isotopes of nitrogen and carbon (expressed as $\delta^{15}N$ and $\delta^{13}C$ respectively) have provided strong evidence for

incorporation of MDN in freshwater food webs. The isotopic composition of adult Pacific salmon, where most individuals range between 10 and 15‰ for $\delta^{15}N$ and between -21 and -19‰ for $\delta^{13}C$ (Welch and Parsons 1993, Satterfield and Finney 2002), is enriched relative to freshwater sources (e.g., Kline et al. 1990). The isotopic disparities between these nutrient pools offers a means for estimating the subsidy of MDN to freshwater food webs (Kline et al. 1990, Bilby et al. 1996, Chaloner et al. 2002, Hicks et al. 2005, Scheuerell et al. 2007). Similar isotopic differences exist between marine and freshwater sulfur (sulfate and organic sulfur), and it is expected that stable sulfur isotopes ($\delta^{34}S$) will track MDN assimilation in aquatic and riparian biota. While sulfur isotope data are few, the $\delta^{34}S$ from adult Pacific salmon ranged between 18 and 20‰ (Stricker, unpublished, n = 20) while values typical of freshwater systems are much lower (e.g., < -10, Hesslein et al. 1991). Sulfur isotopes may offer distinct advantages over carbon and nitrogen. While $\delta^{15}N$ of consumer tissues reflects the nitrogen source, it also indicates trophic position due to stepwise enrichment as a function of trophic level (DeNiro and Epstein 1981, Minagawa and Wada 1984), potentially confounding variations in trophic position with MDN assimilation. Sulfur isotopes, borne on two essential amino acids (methionine and cysteine), are a conservative diet tracer unaffected by trophic position (Hesslein et al. 1991, Barnes and Jennings 2007). In addition to trophic position, $\delta^{15}N$ values in consumers can be influenced by body condition and dietary protein (Gannes et al. 1997).

Fatty acid analysis is another method for understanding food web relationships that can be used to track MDN assimilation in stream food webs. Fatty acids ingested by

consumers, unless directly catabolized for energy, are stored in lipid reserves with little or no modification. As such, the fatty acid composition of a consumer reflects that of its food sources, with individual fatty acids occurring in proportion to that found in the combined food items (Iverson et al. 2004). For some time researchers have capitalized on this phenomenon to understand trophic relationships and the flow of energy in aquatic ecosystems (reviewed by Napolitano 1999, Budge et al. 2006). As with stable isotopes, the relative contribution of multiple food sources to a consumer is best resolved when the food sources have adequately disparate signatures. Production of different polyunsaturated fatty acids by freshwater (including riparian) and marine producers leads to distinct fatty acid signatures for marine and freshwater consumers (Ackman 1999, Napolitano 1999). This disparity is most evident in the relatively high content of ω3 fatty acids relative to ω6 fatty acids in marine fishes; ω3:ω6 ranges from 0.5 to 3.8 in freshwater fish and from 4.7 to 14.4 in marine fish (Henderson and Tocher 1987). Omega3:ω6 for Chinook salmon (*Oncorhynchus tshawytscha*) eggs and flesh (each based on 5 samples from the Anchor River) ranged from 12.1–13.9 and 5.1–7.3, respectively (Ron Heintz, National Marine Fisheries Service, unpublished). The fatty acid signatures of freshwater fish that assimilate MDN come to reflect the marine source, allowing for inferences regarding the contribution of MDN to consumer diets (Heintz et al. 2004, Volk 2004). One main drawback to the use of fatty acids is that, currently, the per-sample cost for laboratory analysis is at least 10-times greater for fatty acids than for stable isotopes.

Although the assimilation of MDN by aquatic and riparian biota and the subsequent benefits to freshwater ecosystems have been established, a better

understanding of the strengths and limitations of different approaches to tracking MDN assimilation, including the extent to which they are influenced by non-MDN variables, would facilitate long-term and spatially extensive monitoring of MDN assimilation. Incorporating MDN budgets into sound fisheries and ecosystem management strategies requires a better understanding of the dose-response relationship between salmon escapement and the ecological benefits of MDN (Wipfli et al. 1999, Bilby et al. 2001, Gende et al. 2002, Wipfli et al. 2003). An emerging line of research has proposed management approaches that rely on an understanding of the quantitative relationship between the stable isotope ratios of stream resident fishes and the abundance of spawning salmon. Bilby et al. (2001) observed an asymptotic relationship between coho salmon (*Oncorhynchus kisutch*) parr $\delta^{15}N$ and spawner abundance, which they proposed as a method for identifying MDN assimilation capacity and, in turn, for setting salmon escapement goals that balance salmon harvest with the ecological demands of salmon streams. In a similar approach, Reichert et al. (2008) indicated that the $\delta^{15}N$ of coho salmon parr could be used to infer the abundance of salmon spawning in streams. While these approaches hold promise for ecologically-based management of salmon streams, accounting for non-MDN factors that may influence or confound measures of MDN assimilation could ultimately increase precision and predictive power. Additionally, it is possible that other measures of MDN assimilation (i.e., fatty acid signatures or sulfur isotopes) or other types of organisms (i.e., riparian plants or aquatic macroinvertebrates) offer advantages over the use of nitrogen isotopes in juvenile salmonids.

The goal of this research was to better understand the influence of spawner abundance and environmental variables (i.e., season, geographic region, fish length, and appropriate interaction terms) on measures of MDN assimilation in stream and riparian species common to the Kenai Peninsula, Alaska. We focused our efforts on stream-resident Dolly Varden (*Salvelinus malma*) due to their near ubiquitous distribution in coastal Alaskan streams, but expanded the emphasis to also include select aquatic macroinvertebrate species (both primary consumers and detritivores) and a common riparian plant (horsetail, *Equisetum fluviatile*). We calculated spawner abundance by two competing methods that corresponded to the following hypotheses: (1) localized spawner density (LSD) is the best predictor of MDN assimilation and (2) the total biomass of spawners upstream of a given station (upstream spawner biomass; USB) is the best predictor of MDN assimilation. LSD corresponds to the hypothesis that MDN assimilation is locally mediated and that, for animals, direct consumption of salmon eggs and/or flesh is the most important uptake pathway. USB corresponds to the hypothesis that downstream transport of MDN is an important process and that food web interactions play a critical role in the downstream transfer of MDN. Additionally, we sought to determine which measures of MDN assimilation (i.e., carbon, nitrogen, and sulfur stable isotopes and fatty acids) were the best predictors of spawner abundance. We evaluated field survey data using Akaike Information Criterion (AIC; Burnham and Anderson 2002) to select the most parsimonious multiple linear regression models expressing stable isotope ratios and fatty acid measures as functions of the above variables.

METHODS

Study sites

We collected field data in three watersheds within each of three regions on the Kenai Peninsula, Alaska (Figure 1). Streams within a given region were in relatively close proximity, were geomorphologically similar to the extent possible (i.e., similar basin area, elevation, channel slope), and contained two salmon spawning streams and one salmon-free reference stream where spawning runs were blocked by waterfalls (Table 1). Within each stream we established three sampling stations: one near the stream mouth, one near the geographic middle of the mainstem, and one in the upper reaches. In salmon streams, the upper station was near the upstream limit of salmon spawning. Due to the lack of road access in most of the watersheds, we generally chose the exact location of sampling based on ease of hiking and/or floatplane access.

Cooper Landing area streams

The Cooper Landing study streams consisted of the Russian River and Quartz Creek as salmon-bearing streams and Juneau Creek as a salmon-free reference (Table 1). These streams are in the Chugach - St. Elias Mountains ecoregion (Nowacki et al. 2001) that has a continental climate with approximately 64 cm of annual precipitation (Brabets et al. 1999). Underlying geology is primarily metamorphic, volcanic, and igneous rock (Brabets et al. 1999) and, as such, these streams had relatively low concentrations of primary nutrients. These streams are within the Kenai River basin, the Kenai Peninsula's largest watershed, which supports substantial sport and commercial salmon fisheries.

These streams are typified by gently sloped mainstem channels that drain mixed spruce (*Picea* spp.) and birch (*Betula papyrifera*) forest and steep tributaries that drain rugged alpine basins.

The Russian River has two mainstem lakes, the Upper and Lower Russian lakes, of which the upper lake serves as a sockeye salmon (*Oncorhynchus nerka*) nursery. Sockeye salmon are the dominant salmon species in this system and two runs spawn each year: over the past decade the early run has averaged ~50,000 fish, most of which pass the weir (located just below Lower Russian Lake) during the second half of June; over the past decade the late run has averaged ~100,000 fish, most of which pass the weir from mid-July to mid-August. The Russian River also receives much smaller numbers of Chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon. During 2004 and 2005, sockeye salmon weir counts were 166,800 and 112,400, respectively (Alaska Department of Fish and Game [ADF&G] data).

Quartz Creek typically receives between 1000 and 20,000 sockeye salmon spawners, although >66,000 were surveyed in 2002 (ADF&G, unpublished data). Quartz Creek, in contrast with the other study streams in the Cooper Landing area, has no major lakes in the basin. As such, sockeye salmon fry that hatch in this system migrate downstream to rear in Kenai Lake. Quartz Creek also receives a small number of Chinook salmon (<100 fish) that spawn prior to the sockeye salmon. The exact timing of Quartz Creek runs is uncertain due to the lack of a weir on this stream, but ADF&G spawner surveys conducted from mid to late August show a mix of live and dead sockeye

salmon at this time, suggesting that this is near peak spawning. Foot surveys during 2004 and 2005 counted 13,500 and 6000 sockeye salmon, respectively

Juneau Creek has a waterfall ~3 km upstream of its confluence with the Kenai River, blocking salmon access to most of the basin. The Juneau Creek basin has two lakes, both of which are located upstream of the falls: Trout Lake is on a tributary to Juneau Creek and Juneau Lake is on the mainstem. All sampling stations on Juneau Creek were located above the falls.

Homer area streams

The Homer-area streams consisted of the North Fork Anchor River and the South Fork Anchor River as salmon-bearing streams and Happy Creek as a salmon-free reference (Table 1). This area is in the Cook Inlet Basin ecoregion (Nowacki et al. 2001) and has a climate transitional between maritime and continental with approximately 57 cm annual precipitation (Brabets et al. 1999). Streams in this area drain extensive unconsolidated glacial deposits and proglacial lake sediments (Selkregg 1974), which results in relatively high ambient nutrient concentrations. These streams drain a rolling landscape of white spruce (*Picea glauca*) forests that have experienced extensive mortality due to the recent spruce bark beetle (*Dendroctonus rufipennis*) outbreak (Rinella et al. 2009). Riparian vegetation is typically mixed cottonwood (*Populus* spp.) and spruce along the lower alluvial stream reaches, giving way to poorly drained soils dominated by willow (*Salix* spp.), bluejoint grass (*Calamagrostis canadensis*), and sedges (*Carex* spp.) in the upper reaches. There are no significant lakes in this area, but

wetlands comprise about 20% of the Anchor and Ninilchik basins (Mauger 2005). The Anchor River supports runs of Chinook and coho salmon, steelhead, and anadromous and resident populations of Dolly Varden.

During 2004, ADF&G's mainstem weir near the mouth of the Anchor River counted 12,000 Chinook salmon from May – July and 5700 coho salmon during August. A companion weir on the lower North Fork Anchor River during 2004 indicated that, of these fish, 1900 Chinook salmon and 700 coho salmon continued up the North Fork, while the remainder spawned in the South Fork. During 2005, the mainstem weir passed 11,100 Chinook salmon and 19,000 coho salmon. Since the North Fork weir did not operate during 2005, we assumed the salmon populations divided themselves between the North and South forks in proportions equal to that observed in 2004. The Happy Valley System has resident Dolly Varden, but no anadromous runs due to the presence of a waterfall at the mouth of the creek.

Seldovia area streams

In the Seldovia region, we sampled the salmon streams Humpy Creek and Barabara Creek, and we sampled China Poot Creek as a salmon-free reference (Table 1). This area is in the Gulf of Alaska Coast ecoregion (Nowacki et al. 2001) and has a maritime climate with approximately 140 cm annual precipitation (Brabets et al. 1999). Underlying geology is primarily metamorphic, volcanic, and igneous rock (Brabets et al. 1999), with some sandstone and shale deposits along Kachemak Bay (Selkregg 1974). As such, these streams had relatively low concentrations of primary nutrients. Streams in

this area are typified by short, steep watersheds that flow directly into Kachemak Bay. Vegetation is dominated by white spruce (*Picea glauca*) and sitka spruce (*Picea sitchensis*), giving way to extensive alder (*Alnus* spp.) stands at higher elevations.

Humpy Creek receives a small run of chum salmon (*Oncorhunchus keta*) that spawn in early August and a sizeable run of pink salmon (*O. gorbuscha*) that spawn during mid to late August. Ground assessments conducted by ADF&G Commercial Fisheries personnel have estimated pink salmon runs of 22,000 to 91,000 over the past five years. Barabara Creek receives runs of pink salmon (averaging 5600 fish based on ADF&G Commercial Fisheries ground assessments) that spawn during late August. China Poot Creek supports a terminal run of sockeye salmon in the lower reaches; however, their access to China Poot Lake, which is situated approximately 2 km above tidewater, is blocked by a waterfall. Sockeye salmon fry are stocked in China Poot Lake and the lake is fertilized regularly to support the fry. We sampled China Poot Creek above the lake, so the terminal fishery and associated management activities did not influence our sampling reach.

Sampling strategy

On each stream we established three sampling stations along the respective drainages extending from stream mouth to headwaters. These stations represented a hypothesized MDN gradient where, within a given stream, the lowermost station had the highest cumulative biomass of upstream spawners and the uppermost station had the lowest. This gradient, coupled with stream-to-stream variation in local spawner

abundance, allowed us to test hypotheses about the relationship between spawner abundance and MDN assimilation in stream and riparian food webs. We sampled each stream prior to the initiation of spawning runs in the spring, during the peak summer spawning period, and (for Cooper Landing and Homer area streams) again during the fall. We were unable to sample Seldovia area streams during the fall because inclement weather restricted aircraft and boat travel. Because each region differed in the dominant spawning salmon species, we shifted summer and fall sampling to ensure that summer sampling coincided with peak salmon spawning and that fall sampling coincided with a period of post-spawn carcass decomposition (Table 2). During each station visit, we collected samples of Dolly Varden, select macroinvertebrate taxa, and horsetail. We collected up to 5 juvenile Dolly Varden per station per sampling event (although this target number was occasionally unmet); when excess Dolly Varden were captured, we retained individuals that spanned the size range observed at the given station. Fork length was measured to the nearest mm and ranged from approximately 50 mm to 150 mm across all streams. We focused on Dolly Varden in this study because this species is ubiquitous in distribution, found in spawning streams and above barriers in salmon-free streams, allowing for the inclusion of salmon-free streams in the model development without the potentially confounding influence of multiple fish species as dependent variables. For macroinvertebrates, we collected dominant taxa of immature aquatic insects representing two trophic levels, taking care to collect the same taxa across all streams when possible. We collected a scraper (i.e., primary consumer; typically the caddidfly *Glossosoma* sp. or the mayfly *Drunella doddsi*), and a shredder (i.e.,

detritivore; typically limnephilid caddiflies *Ecclisomyia conspersa* and *Psychoglypha* sp. or the stonefly *Pteronarcella* sp.). For each taxon, at least 10 individuals were composited to represent a single sample. For horsetail, we gathered 5 individual stems from locations dispersed over the sampling reach; all stems were growing in shallow water or in saturated soil close to the stream margin. We did not collect horsetail during the fall sampling period because it had senesced and we expected this would alter the nitrogen isotopic composition. We selected these plant and animal species to ensure the practicality of the monitoring tools developed during this study: the focal species are likely to be subsidized by MDN when present, wide-spread and common in Kenai Peninsula riverine ecosystems, and relatively easy to collect and identify. We analyzed Dolly Varden and horsetail stems individually but, because of mass constraints on analytical measurements, we analyzed composite samples of macroinvertebrates. All samples were kept on ice in the field or on liquid nitrogen in cases where field storage time exceeded several hours; in the lab, samples were stored in a -70 °C ultra-cold freezer until processed. Fish were homogenized and subsequently split for the stable isotope and fatty acid analyses. Stable isotope samples were oven dried for 48 hours at 65 °C then pulverized. This work was conducted under the University of Alaska Fairbanks IACUC protocol number 06-04.

Laboratory analyses

Carbon and nitrogen stable isotope ratios were measured in Dolly Varden, macroinvertebrates, and horsetail and, additionally, sulfur stable isotopes were measured

in Dolly Varden. Analyses were conducted at the U.S. Geological Survey stable isotope laboratory in Denver, Colorado. Homogenized samples were weighed into tin capsules (5 x 9 mm) and the stable carbon, nitrogen, and sulfur isotopic composition ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively) determined by continuous flow-isotope ratio mass spectrometry. Carbon and nitrogen isotope ratios were measured using a Carlo Erba NC1500 elemental analyzer interfaced to a Micromass Optima mass spectrometer (Fry et al. 1992). Approximately 1-2 mg of vanadium pentoxide (V_2O_5) was added to each tin capsule as a combustion aid for the measurement of sulfur isotope ratios using a Costech ECS4010 elemental analyzer interfaced to a Thermo-Finnigan DeltaPlus XP mass spectrometer (Gieseman et al. 1994). Results are reported in δ-notation as deviations in parts per thousand (‰) relative to a monitoring gas as follows:

$$\delta X = (R_{sample}/R_{standard}) - 1, \qquad \text{(Equation 2.1)}$$

where X is the rare isotope (^{13}C, ^{15}N, or ^{34}S) and R is the appropriate isotope ($^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$) ratio. Carbon and nitrogen isotopic compositions were normalized to USGS 40 ($\delta^{13}C$ = -26.24‰ and $\delta^{15}N$ = -4.52‰) and USGS 41 ($\delta^{13}C$ = 37.76‰ and $\delta^{15}N$ = 47.57‰) and reported relative to internationally accepted scales, V-PDB and Air, respectively. Sulfur isotopic compositions were normalized to NBS 127 (+21.1‰) and IAEA-SO-6 (-34.05‰) and reported relative to the internationally accepted scale, V-CDT. Analytical error was ± 0.2‰ for carbon, nitrogen, and sulfur isotope analyses.

Dolly Varden fatty acid composition was measured at the National Marine Fisheries Service laboratory in Juneau, Alaska. Lipid was extracted from 0.5 to 1.0 g of wet sample homogenate using a modification of Folch's method outlined by Christie

(2003) in a Dionex Accelerated Solvent Extractor (ASE) 200 with 2:1 (v:v) chloroform:methanol. Extracts were washed successively with a 0.88% KCl solution and 1:1 (v:v) methanol:deionized water in a volume equal to 25% of the extract volume to remove co-extractables. Excess solvent was evaporated and percent lipid was calculated gravimetrically. Fatty acids of whole lipid from the Dolly Varden and salmon archetypes were transesterified to fatty acid methyl esters (FAMEs) using Hilditch reagent, as described in Christie (2003) except that hexane was used as a solvent instead of toluene. Prior to transesterification, C19:0 and C23:0 fatty acids were added as an internal standard and a surrogate standard, respectively. Purified FAMEs in hexane were evaporated under nitrogen to a final volume of approximately 1 ml, and a FAME internal standard (C21:0) was added before injection into a Varian CP3800 gas chromatograph (GC). The GC was equipped with a 100 m Varian CP Select for FAME cyanopropyl-bonded fused silica column and operated under a ramped temperature program. Separated fatty acids were detected with a Varian Saturn model 2200 mass spectrometer operating in selective ion storage mode. Samples were processed in batches of 17 along with calibration standards and quality assurance samples. Fatty acid concentrations were determined using five-point calibration curves for each FAME normalized to internal standard recovery. Blank, duplicate and reference (NIST Standard reference material number 1946) sample spectra were used for QA evaluation. Concentrations observed for the reference material were typically within 25% of the certified values. The coefficient of variation for duplicate analyses performed within a batch was generally less than 10%.

The estimated total fatty acid content of method blanks was less than 10% of the lowest estimate for samples in a batch.

We expressed fatty acid compositions in two different ways: (1) as the ratio of ω3 to ω6 (Heintz et al. 2004, Volk 2004) and (2) as the multivariate distance between the fatty acids in a given sample and those measured in the flesh of adult Chinook salmon from the Anchor River (Heintz unpublished). For the latter we used a metric that expresses the distance between two compositions as the sum of the squares of all possible log-ratio differences between the components (Aitchison 1986).

Quantifying spawner abundance

We used 2005 salmon spawner data as predictors for MDN measures in samples collected during summer and fall. Since our spring sampling was conducted prior to the onset of the 2005 spawning runs, we used 2004 spawner data as predictors for these samples. We used data from several ADF&G projects as measures of spawning salmon abundance for the 2004 and 2005 seasons in each of our salmon-bearing study streams. There is likely some error associated with our estimates of spawner abundance, but the wide range observed across the study streams allowed us to meet our research objectives while alleviating the need for extensive and costly spawner surveys.

Cooper Landing area streams

ADF&G operated a counting weir on the Russian River, just below the Russian River Falls, from 9 June –3 September in 2004 and from 7 June –7 September in 2005

(http://www.sf.adfg.state.ak.us/Region2/Escapement/HTML/query.cfm). ADF&G conducted ground surveys to count spawning salmon in the lower 20 km of Quartz Creek during periods of relatively low streamflow. Surveys were conducted on 17 August in 2004 and 24 August in 2005 and targeted peak spawning of the dominant sockeye salmon populations (David Westerman, ADF&G, personal communication).

Homer area streams

On the Anchor River, ADF&G surveyed spawners just above tidewater using a weir coupled with a DIDSON sonar during high-flow periods that rendered the weir ineffective (Kerkvliet et al. 2008). This system operated from 15 May –September 13 in 2004 and 13 May – 9 September in 2005; while it gave overall spawner estimates for the Anchor River system, it did not indicate how these spawners were distributed between the North and South forks (http://www.sf.adfg.state.ak.us/Region2/Escapement/HTML/query.cfm). ADF&G operated an additional weir on the North Fork during 2004 (Kerkvliet et al. 2008), providing counts for the North Fork and, by difference, the South Fork. Since the North Fork weir was not operational in 2005, we assumed salmon species divided themselves between the forks in the same proportion as in 2004.

Seldovia area streams

In Humpy and Barabara creeks, ADF&G conducted periodic ground surveys (5 or 6 surveys per season) with stream life factors of 17.5 days applied to both salmon species

present (pink and chum salmon) (Hammarstrom and Ford 2008; Ted Otis, ADF&G, personal communication).

We calculated the total spawner biomass for each stream (for both 2004 and 2005) using the year-specific average mass for individuals of each species sampled from local commercial catches (Hammarstrom and Ford 2008). The single exception was Anchor River Chinook salmon, for which we used individual mass data from the Anchor River weir (Kerkvliet et al. 2008). We measured the approximate length of each stream used by spawning salmon from ADF&G's interactive Fish Distribution Database (http://www.sf.adfg.state.ak.us/SARR/FishDistrib/FDD_ims.cfm) which enabled stream-specific estimates of spawner biomass densities. In addition to compiling ADF&G data, we conducted ground surveys over a 500-m stream reach at each salmon-bearing station on each sampling date in addition to making opportunistic salmon counts throughout the sampling season. These surveys provided rough estimates of station-to-station variation in spawner densities within each stream.

As independent variables in model selection, we expressed salmon abundance in two different ways that corresponded to our competing hypotheses regarding the influence of salmon abundance on MDN assimilation in stream-resident and riparian biota. Our first MDN abundance measure was localized spawner density (LSD; expressed in MT [metric tons]/km), which we calculated for each station using the stream-specific spawner biomass estimates (calculated with ADF&G data) after adjusting estimates based on ground surveys. At stations where our surveys indicated especially high or low spawner abundance, we adjusted the spawner biomass upward or downward

by 50%; otherwise, we used the estimate derived from ADF&G data. Our second MDN abundance measure was the total biomass of spawners upstream of a given station (USB; expressed in MT). For this measure, we assumed that spawners were evenly distributed over each stream's spawning area so that the spawner biomass at any station was proportional to the extent of available spawning habitat upstream of that point.

Data analysis

Following an information theoretic approach (Burnham and Anderson 2002), we defined a priori a set of linear regression models that corresponded to hypotheses regarding the influence of environmental factors on stable isotope and fatty acid signatures in biota. We then used Akaike Information Criterion adjusted for small sample size (AIC_c) (Akaike 1973, Burnham and Anderson 2002) to select the most parsimonious model from the candidate set. For each dependent variable we report and make inferences using the single best model, as opposed to averaging multiple models, because the models correspond to distinctly different hypotheses regarding the influence of salmon abundance and other environmental factors on MDN assimilation in biota (Burnham and Anderson 2002). Additionally, we were more interested in understanding the influence of salmon abundance and other factors on measures of MDN assimilation than making precise predictions regarding MDN metrics. We used each sampling event within each station as an experimental unit, so we averaged the data from all fish captured at a given station on a given date.

For Dolly Varden, our model set expressed stable isotope ratios and fatty acid signatures as a function of spawning salmon abundance, region, Dolly Varden length, season, and the interactions season x spawner abundance and Dolly Varden length x spawner abundance. Our Dolly Varden model set was identical for all dependent variables and included all combinations of the above independent variables for a total of 53 candidate models. For macroinvertebrates and horsetail, our model set expressed stable isotope ratios as a function of spawning salmon abundance, region, season, and the interaction season x spawner abundance. This model set was also identical for all dependent variables and included all combinations of the above independent variables for a total of 15 candidate models. We included season x spawner abundance interaction terms because we hypothesized that our dependent variables would be relatively constant in salmon-free streams but would vary in response to seasonal fluctuations in MDN availability in salmon-bearing streams. We included the Dolly Varden length x spawner abundance interaction term because we hypothesized that, in salmon-bearing streams, larger Dolly Varden may have a competitive advantage over smaller individuals, thereby assimilating more MDN. For all biota, our candidate models expressed spawning salmon abundance in terms of either local spawner density (LSD) or cumulative upstream spawner biomass (USB) to determine which parameter was a better predictor of MDN assimilation. For each dependent variable, we calculated the relative importance of LSD and USB by summing the Akaike weights (w_i) for all models where each occurred (Burnham and Anderson 2002). Since we were particularly interested in the effect of spawning salmon abundance on our stable isotope and fatty acid measures, we used the

best model and plotted the model estimates for each dependent variable (±95% confidence interval) as a function of spawning salmon abundance; to keep the figures simple, we held constant all parameters that did not interact with salmon abundance. We presented all models that did a reasonably good job of approximating the data (i.e., those where $\Sigma w_i \geq 0.9$). We ran the AIC model selection, estimated the subsequent model parameters, and calculated model estimates using Statistica 6.1 for Windows. We omitted carbon stable isotopes of horsetail from analysis because terrestrial plants assimilate carbon from the atmosphere.

RESULTS

Spawner abundance

Across all salmon-bearing stations, LSD ranged from 0.3 – 22 MT/km in 2004 and from 0.3 – 23 MT/km in 2005 while USB ranged from 0.8 – 382 MT in 2004 and from 0.6 – 222 MT in 2005 (Table 3). Within their respective regions, stations in the Russian River, S.F. Anchor River, and Humpy Creek had the highest LSD and USB.

Dolly Varden stable isotopes

For brevity, we present model estimates for only the Cooper Landing region throughout the results section. Based on AIC_c, the best model for Dolly Varden $\delta^{15}N$ was $y = LSD + region + DV\ length$ (Table 4). This model estimates $\delta^{15}N$ for the smallest Dolly Varden (~50 mm) in the salmon-free Cooper Landing stream to be 6.7±0.7‰ (β_0 ± standard error). Estimates were slightly higher in the Homer region (β_{Homer} = 0.4±0.4‰)

and lower in the Seldovia region ($\beta_{Seldovia}$ = -2.0±0.5‰). Delta ^{15}N increased slightly with Dolly Varden length (β_{length} = 0.01±0.006‰) and substantially with LSD (β_{LSD} = 0.23±.028‰) (Figure 2). This model explained 66% of the variation in Dolly Varden $\delta^{15}N$. Aside from the parameters in the best model, our data gave some support for a Dolly Varden length x LSD interaction and a season effect (Table 4). Summing the w_i values across the entire model set indicated that LSD was clearly a better predictor of $\delta^{15}N$ than was USB ($\Sigma w_i > 0.9999$ and < 0.0001, respectively).

The best model for Dolly Varden $\delta^{13}C$ was $y = USB + region + DV\ length$ (Table 4). This model predicts $\delta^{13}C$ in the smallest Dolly Varden in the salmon-free Cooper Landing stream to be -29.6±1.5‰ (β_0±standard error). Delta ^{13}C was lower in the Homer and Seldovia regions (β_{Homer} = -2.6±0.8‰, $\beta_{Seldovia}$ = -2.4±1.1‰) and increased with Dolly Varden length (β_{length} = 0.03±0.01‰) and with upstream spawner biomass (β_{USB} = 0.02±0.01‰) (Figure 3). This model described relatively little variation in Dolly Varden $\delta^{13}C$ (multiple R^2 = 0.33). In addition to the parameters in the best model, our data showed evidence for the effect of LSD (Table 4), but USB was a somewhat better predictor of $\delta^{13}C$ (Σw_i = 0.36 and 0.63, respectively). Our data also gave some support for an interaction between Dolly Varden length and spawner abundance and for a season effect (Table 4).

For Dolly Varden $\delta^{34}S$, our data were best approximated by the model $y = LSD + region + DV\ length + (LSD \times DV\ length)$ (Table 4). The smallest Dolly Varden from the salmon-free Cooper Landing stream were predicted to have $\delta^{34}S$ of 1.1±1.7‰ (β_0 ± standard error). Delta ^{34}S was higher in the Homer region (β_{Homer} = 10.0±0.9‰), lower in

the Seldovia region ($\beta_{Seldovia}$ = -4.7±1.2‰), and increased with Dolly Varden length (β_{length} = 0.001±0.02‰). Values decreased slightly with LSD (β_{LSD} = -0.3±0.3‰) although there was an interaction between Dolly Varden length and LSD ($\beta_{length \times LSD}$ = 0.01±0.003‰) (Figure 4). This model explained 83% of the variation in Dolly Varden $\delta^{34}S$. Our data also gave some support for a season effect as well as a season x LSD interaction (Table 4). LSD was clearly a better predictor than USB ($\Sigma w_i > 0.9999$ and < 0.0001, respectively).

We have presented selected raw data plots ($\delta^{15}N$ and $\delta^{34}S$ with special reference to the Cooper Landing region) to support the modeling results described here (Figures 5 and 6). Panel A of each figure shows the entire set of isotope data for the three regions, each data point representing the average value for fish at a given station. There is considerable variation in both nitrogen and sulfur isotopic values, especially at low spawner density, and the $\delta^{34}S$ data show little sign of a positive correlation with spawner density. As Panel B of Figures 5 and 6 show, clearer patterns emerge when the data are partitioned into regions (the Cooper Landing region, in this case) and, additionally, the effect of Dolly Varden length becomes apparent (i.e., fish >100 mm vs. fish <100 mm). These plots clearly support the effect of LSD, region, and Dolly Varden length on nitrogen and sulfur isotope ratios and, in the case of sulfur, the interactive effect of Dolly Varden length and LSD is apparent.

Dolly Varden fatty acids

The best model for Dolly Varden ω3:ω6 fatty acids was $y = LSD + region + DV\ length + (LSD \times DV\ length)$ (Table 5). Omega 3:ω6 was predicted to be 4.6±1.3 (β_0 ± standard error) for small fish in salmon-free Cooper Landing streams, and values were lower in the Homer region (β_{Homer} = -1.8±0.7) and slightly higher in the Seldovia region ($\beta_{Seldovia}$ = 0.1±0.9). Omega 3:ω6 increased slightly with Dolly Varden length (β_{length} = 0.004±0.1) and decreased with LSD (β_{LSD} = -0.4±0.2), although there was an interaction between Dolly Varden length and LSD where ω3:ω6 responded more positively to LSD among larger Dolly Varden (β_{length} x LSD = 0.01±0.002) (Figure 7). This model explained 44% of the variation in Dolly Varden ω3:ω6. Although LSD was the spawner abundance metric in the top model, our data indicated that, overall, USB was a better predictor (Σw_i = 0.38 and 0.61, respectively). Our data also gave some support to a length x USB interaction and a season effect (Table 5).

For fatty acid distance from salmon flesh, the best model was $y = LSD + region + DV\ length$ (Table 5). This model predicted the distance metric to be 38.5±3.0 (β_0 ± standard error) for small fish in salmon-free Cooper Landing streams. The distance metric was greater in Homer (β_{Homer} = 12.3±1.7) and Seldovia ($\beta_{Seldovia}$ = 9.9±2.1) and it decreased with Dolly Varden length (β_{length} = -0.04±0.03) and with LSD (β_{LSD} = -0.4±0.1) (Figure 8). This model explained 64% of the variation in the distance metric. In addition to the parameters in the best model, our data showed support for a season effect, a length x LSD interaction, and USB. However, LSD was a better predictor than USB (Σw_i = 0.84 and 0.13, respectively).

Macroinvertebrate and horsetail stable isotopes

Our shredder $\delta^{15}N$ data was best approximated by the model $y = LSD + season + region + (season \times LSD)$ (Table 6). Shredder $\delta^{15}N$ was predicted at 2.8±0.5‰ (β_0 ± standard error) for spring samples in salmon-free Cooper Landing streams. Delta^{15}N was higher in Homer streams (β_{Homer} = 1.3±0.5‰), lower in Seldovia streams ($\beta_{Seldovia}$ = -2.6±0.6‰), increased with LSD (β_{LSD} = 0.1±0.05‰) (Figure 9), and increased during summer and fall (β_{summer} = 0.6±0.6‰, β_{fall} = 0.1±0.6‰). Delta^{15}N responded more strongly to LSD in the summer and fall than in the spring ($\beta_{summer \times LSD}$ = 0.1±0.08‰, $\beta_{fall \times LSD}$ = 0.2±0.1‰) (Figure 9). LSD, with $\Sigma w_i > 0.99$, was clearly a better predictor than USB. The above model explained 58% of the variation in shredder $\delta^{15}N$.

The best model for shredder $\delta^{13}C$ was $y = USB + season + (season \times USB)$ (Table 6). Shredder $\delta^{13}C$ was estimated at 29.4±0.7‰ (β_0 ± standard error) for salmon-free stations in spring and was less in the summer and fall (β_{summer} = -1.3±1.0‰, β_{fall} = -1.2±1.0‰). Overall, $\delta^{13}C$ increased slightly with USB (β_{USB} = 0.004±0.006‰) but summer and fall samples rose more steeply ($\beta_{summer \times USB}$ = 0.03±0.01‰, $\beta_{fall \times USB}$ = 0.04±0.01‰) (Figure 10). Compared to top models for most other MDN metrics, this model explained relatively little variation (multiple R^2 = 0.35). In addition to the above parameters, our data supported a region effect (Table 6). USB was clearly a better predictor of shredder $\delta^{13}C$ than was LSD (Σw_i = 0.97 and 0.032, respectively).

Our scraper $\delta^{15}N$ data were best described by the model $y = USB + region$ (Table 6). This model showed $\delta^{15}N$ to be 3.2±0.4‰ (β_0 ± standard error) in salmon-free Cooper Landing streams, to be higher in Homer (β_{Homer} = 3.2±0.5‰), lower in Seldovia ($\beta_{Seldovia}$

= -1.9±0.7‰), and to increase with USB (β_{USB} = 0.01±0.003‰, Figure 11). This model explained 66% of the variation in scraper $\delta^{15}N$. USB was a much better predictor than LSD (USB $w_i > 0.99$). In addition to the top model's parameters, our data supported a season effect (Table 6).

The best model for our scraper $\delta^{13}C$ data was $y = USB + season + region$ (Table 6). This model predicted scraper $\delta^{13}C$ at salmon-free Cooper Landing stations to be -34.7‰ during spring, for $\delta^{13}C$ to be lower in Homer and Seldovia (β_{Homer} = -2.5±1.0‰, $\beta_{Seldovia}$ = -0.04 ± 1.4‰), and for $\delta^{13}C$ to increase with USB (β_{USB} = 0.02±0.006‰, Figure 12) and during summer and fall (β_{summer} = 2.5±1.0‰, β_{fall} = 0.4±1.4‰). This model described relatively little variation in scraper $\delta^{13}C$ (multiple R^2 = 0.35). USB was a much better predictor than was LSD (w_i = 0.98 and 0.013, respectively).

The best model for horsetail $\delta^{15}N$ was $y = LSD + season + region$ (Table 6). The estimate for horsetail $\delta^{15}N$ at salmon-free Cooper Landing stations during spring was 1.6±0.6‰ and was higher in Homer (β_{Homer} = 0.8±0.7‰) and lower in Seldovia ($\beta_{Seldovia}$ = -2.6±0.7‰). Delta^{15}N increased with LSD (β_{LSD} = 0.2±0.04‰, Figure 13) and during summer (β_{summer} = 0.7±0.5‰). This model explained 50% of the variation in horsetail $\delta^{15}N$. LSD, with $\Sigma w_i > 0.9999$, was clearly a better predictor than USB. In addition to the above parameters, our data support a season x LSD interaction (Table 6).

DISCUSSION

The effect of spawner abundance on marine-derived nutrient metrics

Spawner abundance, whether expressed as LSD or USB, was an important predictor for all MDN metrics, and the best model for each MDN metric contained a measure of spawner abundance. This result confirms the assimilation of MDN by multiple trophic levels of stream and riparian ecosystems and indicates the ability of stable isotope and fatty acid measures to reliably detect MDN assimilation within watersheds. This confirmation is strengthened by the independent and similar responses of stable isotopes and fatty acids, reflecting MDN assimilation into proteins and lipid stores, from a common set of Dolly Varden. At the highest salmon abundance, stable isotopes and ω3:ω6 of Dolly Varden approached that of spawning salmon while stable isotopes of macroinvertebrates and horsetail were generally more depleted than spawning salmon

While neither LSD nor USB was the better predictor variable for all taxa, some generalizations can be made about the role of these spawner abundance measures and the hypotheses they represent. USB was the better predictor of scraper nitrogen and carbon stable isotopes (w_i = 0.99 and 0.97, respectively). Scrapers consume epilithic periphyton, composed primarily of diatoms and protists that assimilate these elements as dissolved constituents in stream water. Therefore, the total biomass of spawners upstream of a given point would have a strong influence on the downstream transport of MDN, the isotopic composition of available nutrients and, in turn, the isotopic composition of periphyton and the animals that consume it. While isotope ratios for periphyton would be

instructive, we did not collect samples due to the difficulties associated with isolating algal cells and inherent isotopic variance associated with in-stream primary producers (Finlay et al. 1999).

LSD was by far the better predictor for horsetail $\delta^{15}N$ ($w_i = 1.0$). Downstream nitrogen transport could conceivably be an important mechanism for this species as well (O'Keefe and Edwards 2002), but this result suggests that the effect of salmon carcasses on horsetail was localized. Salmon carcasses transported to the riparian zone by high water and/or scavengers, events commonly observed at those stations with abundant spawners, may have contributed significantly to the nitrogen budget of horsetail. Hilderbrand et al. (1999) estimated that brown bears (*Ursus arctos*) redistribute as much as 5.1 mg m^{-2} of salmon-derived nitrogen into riparian zones of Kenai Peninsula streams. Also, experimental releases of tagged coho salmon carcasses indicated that carcasses were generally retained near their point of release (median distance drifted was 50 m) and that many carcasses ultimately settled on streambanks (20–89% depending on stream and tagging method) (Cederholm et al. 1989).

For Dolly Varden and shredders, LSD was clearly the better predictor for $\delta^{15}N$ (w_i = 1.0 and 0.99, respectively) and for $\delta^{34}S$ ($w_i = 1.0$ for Dolly Varden). Conversely, USB was clearly the better predictor for shredder $\delta^{13}C$ ($w_i = 0.97$) and marginally better for Dolly Varden $\delta^{13}C$ ($w_i = 0.63$). Why nitrogen and sulfur isotopes reflected LSD while carbon isotopes reflected USB is not clear. We did not extract lipid from our samples, so $\delta^{13}C$ patterns may have been influenced by variation in lipid content (Sotiropoulos et al. 2004). Another recent study has noted that, relative to nitrogen isotopes, carbon isotopes

were less influenced by the abundance of spawning salmon (Reichert et al. 2008). For Dolly Varden fatty acids, LSD was the better predictor for fatty acid distance from salmon flesh (w_i = 0.85) while USB was marginally the better predictor for ω3:ω6 (w_i = 0.61), indicating that fatty acid composition was related to spawner abundance but not clearly driven by one spawner abundance measure. Analysis of stomach contents led Scheuerell et al. (2007) to conclude that direct consumption of eggs and flesh was the most important pathway for MDN assimilation in rainbow trout (*Oncorhynchus mykiss*). We attempted to characterize Dolly Varden stomach contents for this study, but overnight trapping was typically required to get adequate samples and any stomach contents were digested by morning. Numerous papers have reported the direct consumption of salmon tissue by shredding macroinvertebrates, especially limnephilid caddisflies (Kline et al. 1990, Piorkowski 1995, Minakawa and Gara 1999, Windler et al. 2005, Walter et al. 2006), and we observed the same phenomenon during this study.

The effects of other factors on marine-derived nutrient metrics

Our results also show that, while spawner abundance is an important predictor, other factors also contribute to variation in MDN metrics. Region was an important predictor for every MDN metric, suggesting that regions have unique backgrounds and that salmon effects are additive upon these background values. Nitrogen isotopes most clearly demonstrate this phenomenon: for all taxa considered in this study, nitrogen isotope values were highest in Homer streams, moderate in Cooper Landing, and lowest in Seldovia at a given spawner abundance (including zero salmon). Scraper

macroinvertebrates hold the lowest trophic position and should, therefore, most closely reflect the dissolved nitrogen pool (Cabana and Rasmussen 1996). Scraper $\delta^{15}N$ from salmon-free stations was 6.9±1.5 (mean ± standard deviation) in the Homer region, 2.7±1.5 in Cooper Landing, and -0.19±0.45 in Seldovia. The basis for these regional differences is not clear, but a few potentially additive causes stemming from differences in watershed nitrogen sources (Peterson and Fry 1987, Cabana and Rasmussen 1996) or subsequent isotopic fractionation of the nitrogen pool can be postulated. One influence may be differences in the extent of alder cover which, through nitrogen fixation, can contribute significant quantities of atmospheric (i.e., 0‰) nitrogen to riparian ecosystems (Helfield and Naiman 2002). Another factor could be differences in denitrifying potential of riparian soils, where fine-textured soils along meandering stream reaches can support high denitrification potential and, in turn, enrichment of the dissolved nitrate pool (Pinay et al. 2003). Lastly, water chemistry samples from salmon-free stations (unpublished data) indicated that primary production may have been limited by nitrogen availability in the Homer streams (total molar N:P = 17) but not in the other regions (total molar N:P > 100), possibly leading to discriminatory assimilation of the lighter isotope by primary producers at Cooper Landing and Seldovia (Teranes and Bernasconi 2000, Brahney et al. 2006). Similar differences in source and fractionation likely explain regional differences in baseline sulfur and carbon isotopes while differences for regional baselines in fatty acid composition may stem from differences in the composition of algal (Ahlgren 1992, Torres-Ruiz 2007), macroinvertebrate (Hanson et al. 1985), or riparian plant communities (Volk 2004).

For all Dolly Varden MDN metrics, fish length was an important predictor. Longer Dolly Varden were associated with increased MDN assimilation in all top models, especially for $\delta^{13}C$ and fatty acid distance from salmon flesh. Top models for $\delta^{34}S$ and $\omega3$:$\omega6$ fatty acids indicated interactive effects, where the influence of spawner abundance was negligible for the smallest Dolly Varden (~50 mm) but substantial for the largest (~150 mm), and the remaining MDN metrics for Dolly Varden had at least some support for similar interactions. These results indicate size-mediated differences in MDN assimilation where larger Dolly Varden appear to consume more MDN than smaller individuals and, for those metrics with interactions, that the smallest Dolly Varden assimilated little or no MDN. This differential MDN assimilation may be due to intraspecific competition where larger individuals are better able to compete for prime feeding positions below salmon redds from which to capture drifting eggs and flesh. Additionally, larger individuals are inevitably released from gape limitation constraints that may prevent smaller individuals from consuming larger pieces of flesh. Gape limitation is not expected to prevent Dolly Varden of the size used in this study from consuming salmon eggs, except possibly in the case of the smallest individuals. Gape measurements from 34 Dolly Varden showed that even the smallest individual (55 mm) had a gape equal to the maximum diameter of Alaskan salmon eggs (i.e., 7 mm for the largest Chinook and chum salmon eggs; Morrow 1980). Without diet and behavioral data these explanations are speculative and further studies are required to determine the mechanism for the observed size-dependent responses. Scheuerell et al. (2007), working with rainbow trout substantially larger than the Dolly Varden in the current study (100–

2000 g vs. 0.4–70 g), found that $\delta^{15}N$ increased with body size but that summer diets for trout of all sizes contained similarly high proportions of salmon eggs and flesh (i.e., trout size had no effect on $\delta^{15}N$ of the summer diet). They concluded that larger fish required larger amounts of non-MDN food sources (presumably in the fall through spring) to shift their isotopic signatures away from a salmon signal. They also found no relationship between body size and $\delta^{15}N$ for sympatric arctic grayling (*Thymallus arcticus*) that consumed primarily macroinvertebrates.

No top models for Dolly Varden MDN metrics included a season effect and, with the exception of $\delta^{34}S$ ($\Sigma w_i = 0.59$), any other models with season as a factor had relatively low model weights (i.e., $\Sigma w_i < 0.1$). These results indicate that season had little or no effect on most measures of MDN assimilation in Dolly Varden, which is somewhat remarkable given that spring samples followed the previous spawning run by ~6 months. In a recent study that examined seasonal patterns in MDN metrics, Reichert et al. (2008) found that $\delta^{15}N$ of juvenile coho salmon from Western Washington streams increased coincident with fall/winter coho salmon spawning (November through January) and then decreased during the rest of the year. Such seasonal variations, or lack thereof, could be explained by differences in the timing of salmon spawning and water temperature regimes. In laboratory and field studies with whitefish (*Coregonus* spp.) muscle, $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ reflect summer growth year-round, and tissue turnover during fall and winter was negligible (Hesslein et al. 1993, Perga and Gerdeaux 2005). Growth potential in fish is tightly linked to water temperature (Elliott 1975) and, based on the growth equations in Jobling (1983), the growth rate for 100-mm Arctic char (*Salvelinus alpines*)

fed on maximum rations is slight at 0 °C (0.036% day^{-1}), increases by an order of magnitude at 4 °C (0.53% day^{-1}), and peaks at 14 °C (1.75% day^{-1}). Western Washington has winters mild enough to permit year-round growth of juvenile coho salmon; for example, coho salmon parr more than doubled their weight between fall and spring in Big Beef Creek where water temperatures rarely fall below 4 °C (Quinn and Peterson 1996). Likewise, Bilby et al. (1996) found that winter growth rates for juvenile coho salmon more than doubled (i.e., from 7 mg $fish^{-1}$ day^{-1} to 16 mg $fish^{-1}$ day^{-1}) in the winter months following salmon spawning. Delta ^{15}N in the coho salmon parr studied by Reichert et al. (2008) indicated substantial MDN assimilation during the winter months with a shift to assimilation of non-MDN food sources during the rest of the year facilitated by warmer water temperatures and increased potential for growth. In Kenai Peninsula streams, by contrast, most salmon spawning occurs from June through October, coincident with relatively warm water and the highest seasonal growth potential. Here, Dolly Varden assimilate MDN during this time and, soon afterward, water temperatures fall to 0 °C and remain there through April (Sue Mauger, Cook Inlet Keeper, unpublished data; Kyle and Brabets 2001). During this time, fish metabolism slows and growth all but ceases, presumably maintaining the MDN signal in Dolly Varden tissues throughout the cold winter months (Hesslein et al. 1993, Perga and Gerdeaux 2005) and until spring, when warming waters in spring are soon followed by renewed availability of MDN resources. It is not clear why $\delta^{34}S$ showed evidence of a season effect while $\delta^{15}N$ and $\delta^{13}C$ did not, especially since these three elements appear to have similar turnover rates (Hesslein et al. 1993).

Contrary to those for Dolly Varden, top models for macroinvertebrates and horsetail showed that MDN signatures varied seasonally. The sole exception was scraper $\delta^{15}N$, which showed only limited support for a season effect ($\Sigma w_i = 0.32$). Relative to spring samples, scraper $\delta^{13}C$ was higher during summer and slightly higher during fall. Horsetail $\delta^{15}N$ was also higher during summer than spring (horsetail was not sampled during the fall due to senescence). Shredder $\delta^{15}N$ and $\delta^{13}C$ showed interactions where summer and fall samples had a greater response to spawner abundance than spring samples. For macroinvertebrates, this pattern may be related to rapid tissue turnover (Cabana and Rasmussen 1996) and their relatively short life cycles. While little is known regarding the life history of macroinvertebrates in south-central Alaska, the scrapers and shredders sampled tended to be large-bodied taxa (i.e., *Drunella*, *Glossosoma*, Limnephilidae) that probably have univoltine life cycles (Irons 1988). Annual emergence acts to reset the chemical signatures of these organisms, and our data suggest the chemical makeup of macroinvertebrates was influenced by the abundance of spawning salmon during the time leading up to sampling. A similar mechanism is likely for horsetail which, although it is a perennial, grows new stems each year.

Recommendations for watershed marine-derived nutrient monitoring

Reichert et al. (2008) recently proposed capitalizing on $\delta^{15}N$ enrichment in juvenile coho salmon as a tool to estimate the abundance of spawning salmon in streams. They found a positive linear relationship between carcass availability and juvenile coho salmon $\delta^{15}N$, with 45% of the variation in $\delta^{15}N$ explained. This is a promising

management application for MDN tracking techniques and, if potentially confounding variables can be identified and accounted for, a more precise underlying relationship between spawner abundance and MDN measures should emerge. Our study represents a step in that direction.

The utility of any metric of MDN assimilation is indicated by effect size and precision of the model estimates (i.e., the slope of the line and the width of the error bars). Of the MDN metrics considered in our study, Dolly Varden $\delta^{15}N$ and $\delta^{34}S$ offered the best combination of effect size and precision, with 95% confidence intervals of the top models offering the best resolution of LSD across the range observed in this study. We hypothesize that this outcome is related to the mobility of Dolly Varden which permits them to move as necessary to capitalize on patchily-distributed MDN resources. Aside from the observed higher precision and effect size, stable isotopes offer the added advantage of significantly lower analytical costs relative to fatty acid analyses. Recent per-sample cost estimates are at least 10 times greater for fatty acids than for stable isotopes. It is also worth noting that nitrogen isotopes were consistently better predictors than carbon isotopes, explaining up to twice the variation in spawner abundance for all taxa where both isotopes were modeled (i.e., Dolly Varden, shredders, and scrapers).

Our top models for $\delta^{15}N$ and $\delta^{34}S$ included factors for region and Dolly Varden length in addition to LSD, and these models explained 66% and 83% of the variation in $\delta^{15}N$ and $\delta^{34}S$, respectively. With these covariates, our models offered a substantial improvement over Reichert et al.'s (2008) bivariate models (R^2=0.45). Region and Dolly Varden length should be taken into account when making inferences about spawner

abundance based on MDN measures, especially when collecting data over broad spatial scales or from fish of variable sizes. Season was not a good predictor of any MDN metric, suggesting that MDN signals in Dolly Varden are maintained year-round in south-central coastal Alaskan streams and that the timing of sampling may not be of critical importance.

The results of this study show that both fatty acids and stable isotopes are reliable indicators of salmon spawner presence and abundance in streams, and that both show promise as tools for monitoring anadromous fish abundance and MDN assimilation in watersheds. Stable isotopes may be more practical because they are easier to sample and handle, cheaper to process and analyze, and because data are less complicated to interpret. The most salient outcome of this work is confirmation of the ability to estimate the salmon spawner density in a stream, within a known margin of error, based on the isotopic signatures of juvenile fish rearing there.

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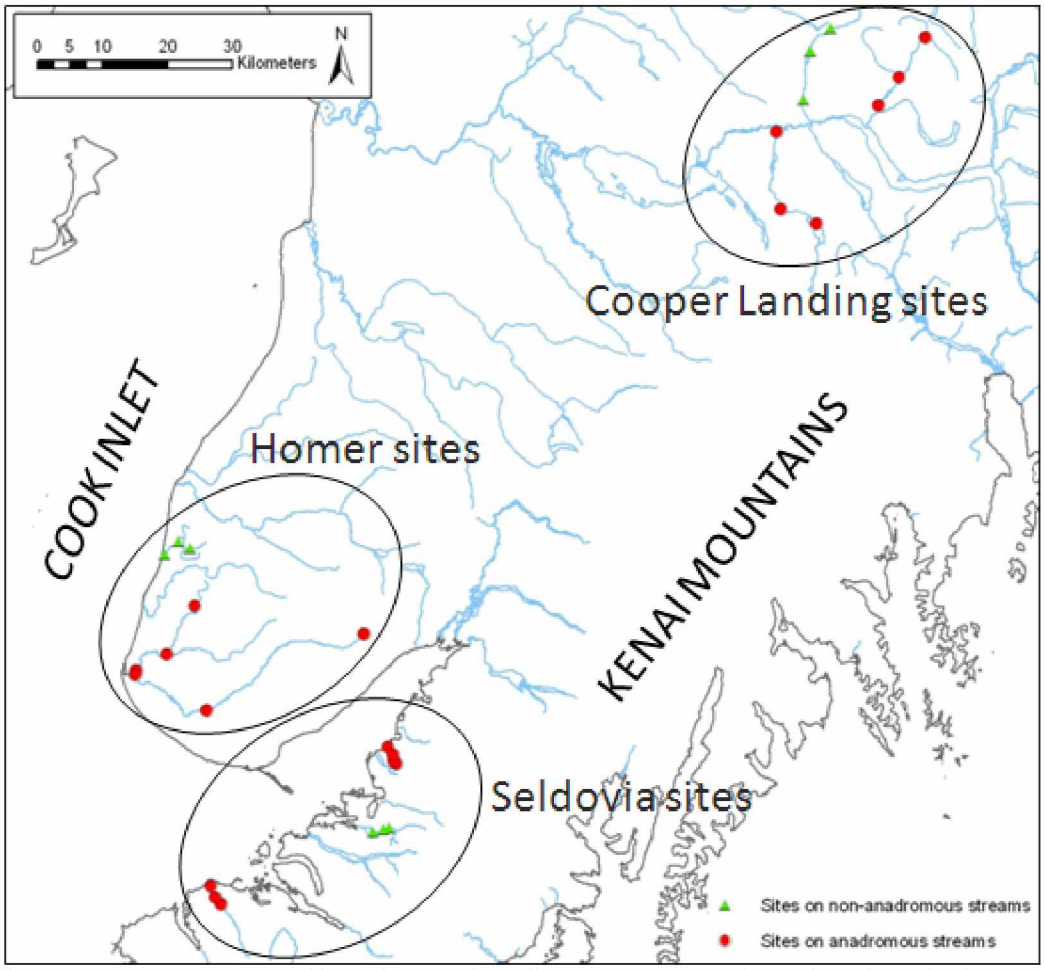

Figure 2.1. Stream sampling sites and stations on the Kenai Peninsula, Alaska.

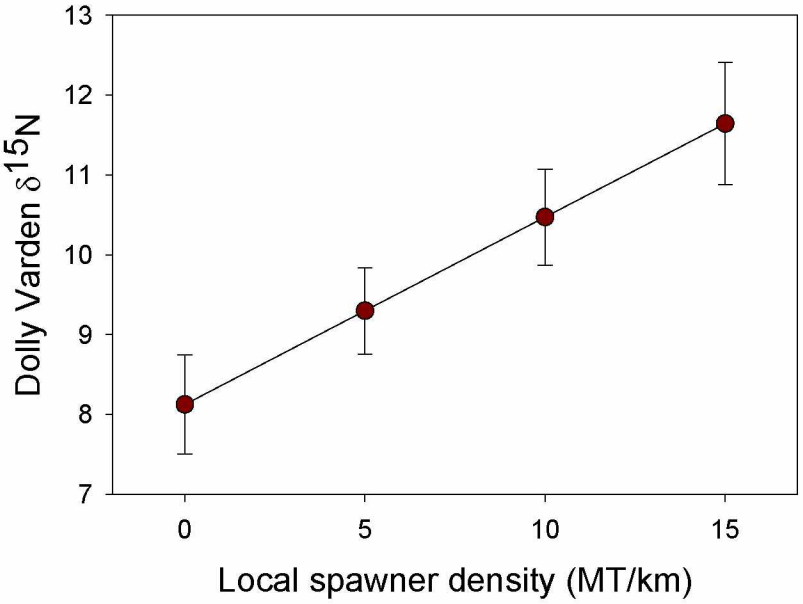

Figure 2.2. Model estimates for Dolly Varden $\delta^{15}N$ (± 95% CI) across 4 levels of local spawner density. Estimates are for 100-mm Dolly Varden from the Cooper Landing region. MT=metric tons.

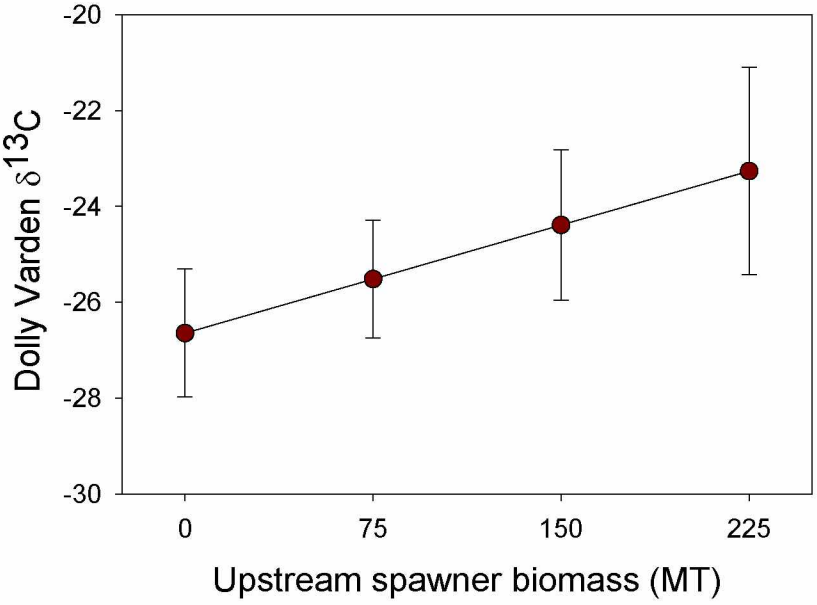

Figure 2.3. Model estimates for Dolly Varden $\delta^{13}C$ (± 95% CI) across 4 levels of upstream spawner biomass. Estimates are for 100-mm Dolly Varden from the Cooper Landing region. MT=metric tons.

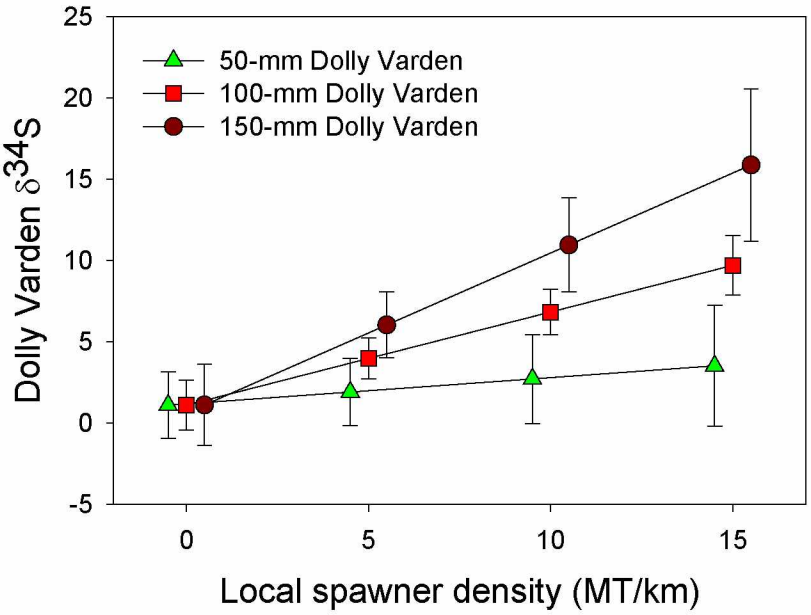

Figure 2.4. Model estimates for Dolly Varden $\delta^{34}S$ (± 95% CI) across 4 levels of local spawner density. Estimates are for Dolly Varden of 3 different lengths from the Cooper Landing region. MT=metric tons.

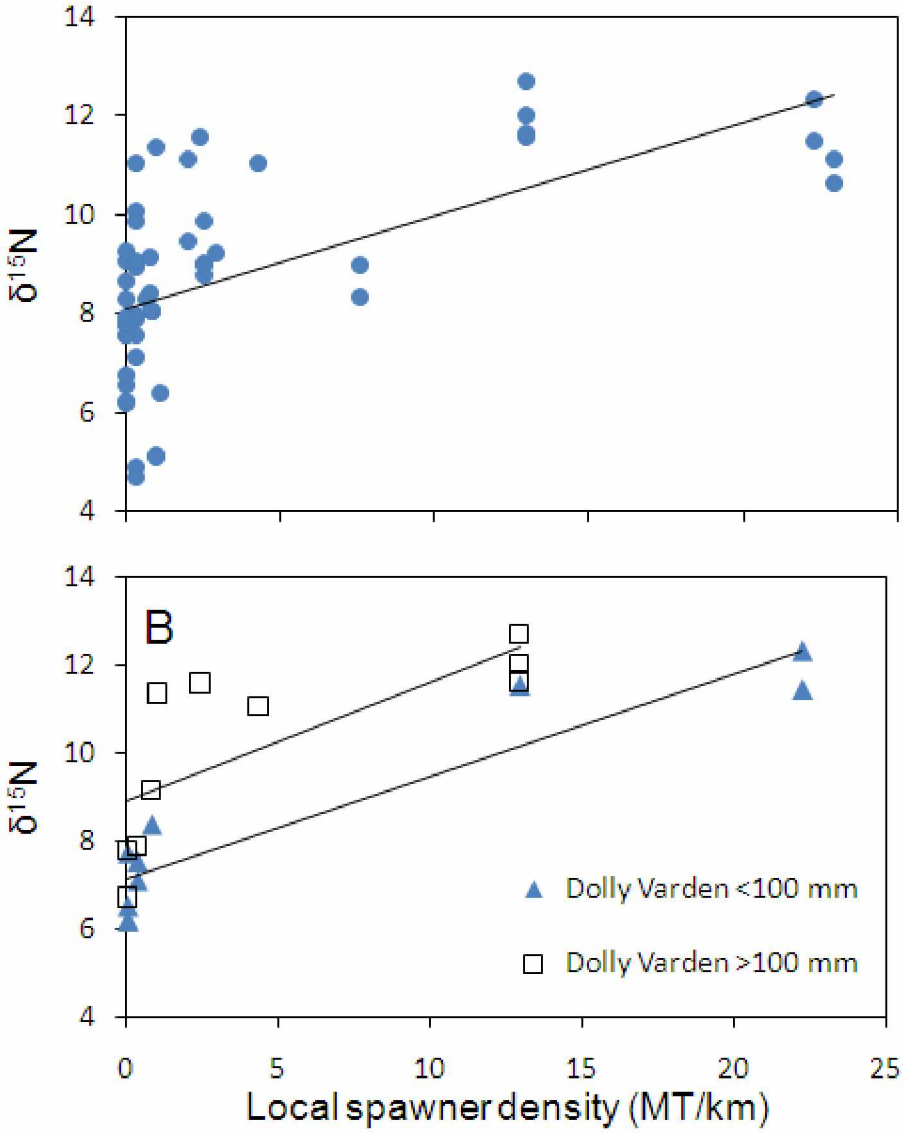

Figure 2.5. Raw $\delta^{15}N$ data as a function of local spawner density for all sites (Panel A) and for Cooper Landing sites (Panel B). MT=metric tons.

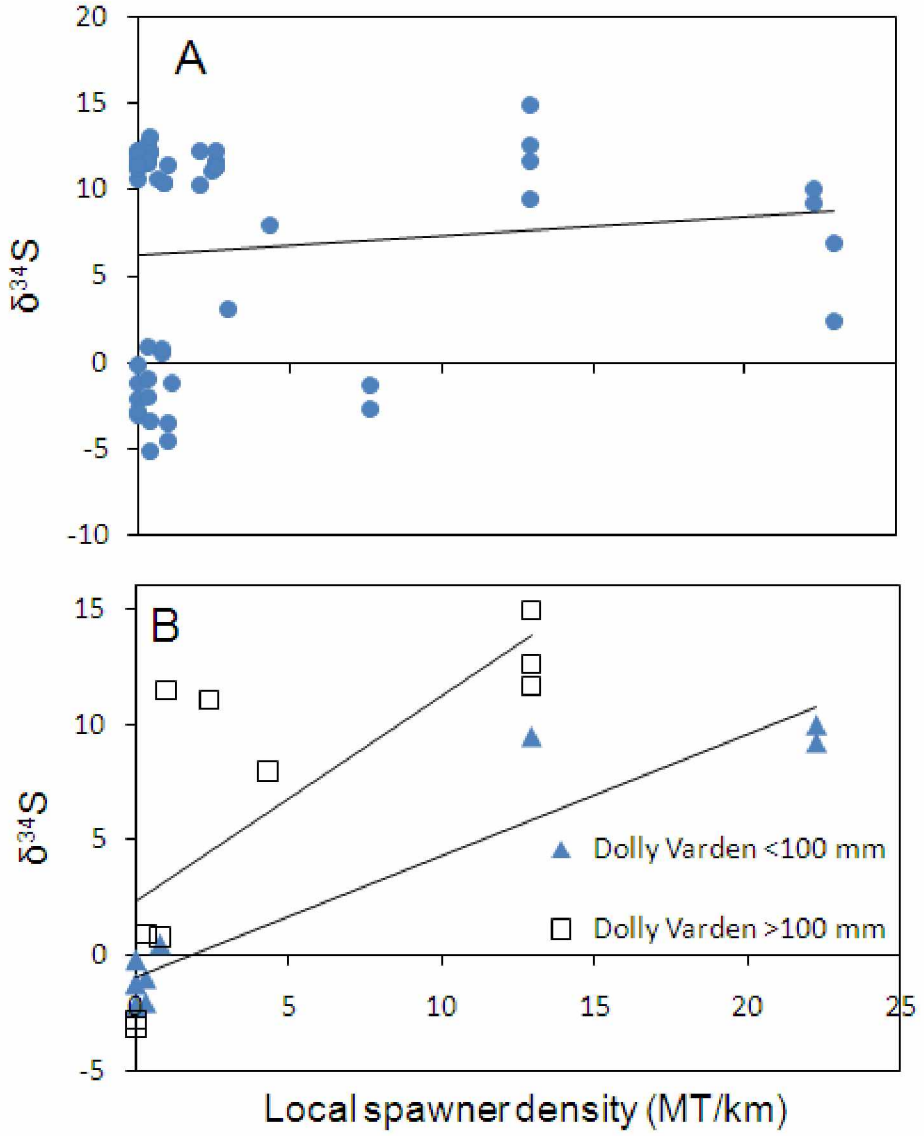

Figure 2.6. Raw $\delta^{34}S$ data as a function of local spawner density for all sites (Panel A) and for Cooper Landing sites (Panel B). MT=metric tons.

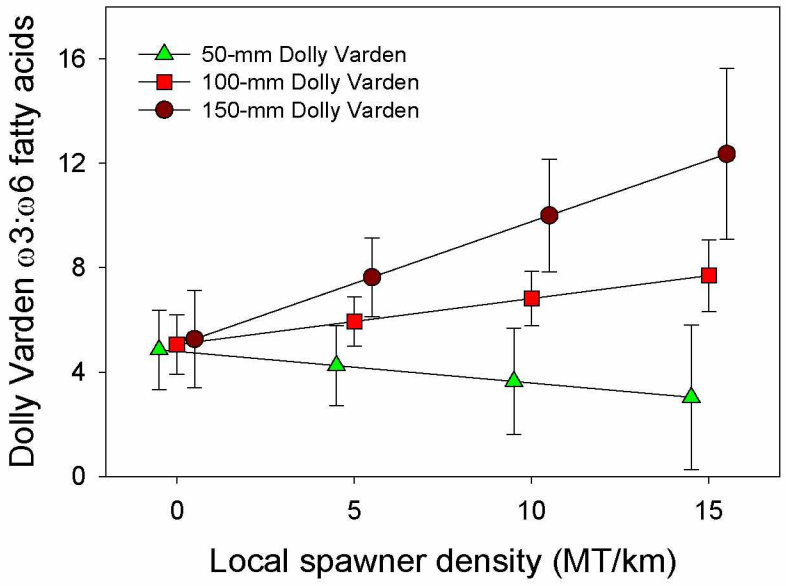

Figure 2.7. Model estimates for Dolly Varden ω3:ω6 fatty acids (± 95% CI) across 4 levels of local spawner density. Estimates are for Dolly Varden of 3 different lengths from the Cooper Landing region. MT=metric tons.

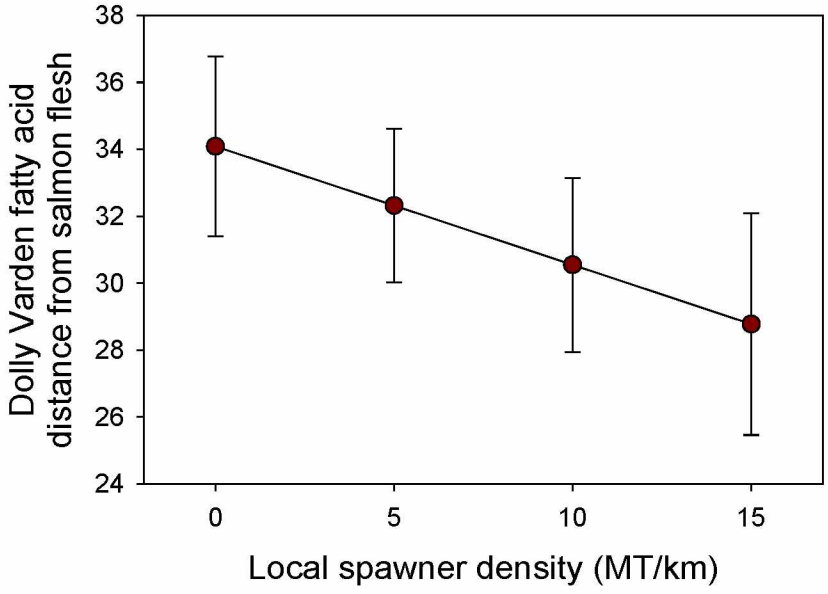

Figure 2.8. Model estimates for Dolly Varden fatty acid distance from salmon flesh (± 95% CI) across 4 levels of local spawner density. Estimates are for 100-mm Dolly Varden from the Cooper Landing region. MT=metric tons.

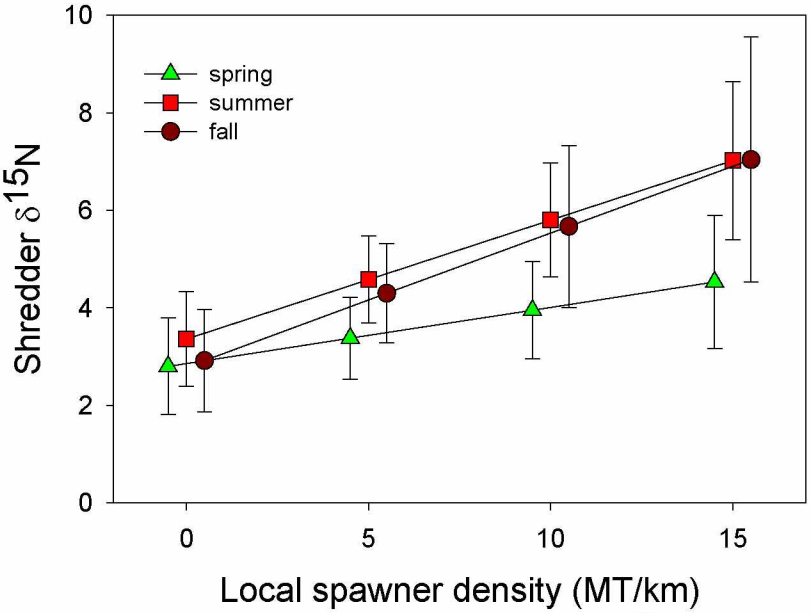

Figure 2.9. Model estimates for shredder macroinvertebrate $\delta^{15}N$ (± 95% CI) across 4 levels of local spawner density. Estimates are for the Cooper Landing region. MT=metric tons.

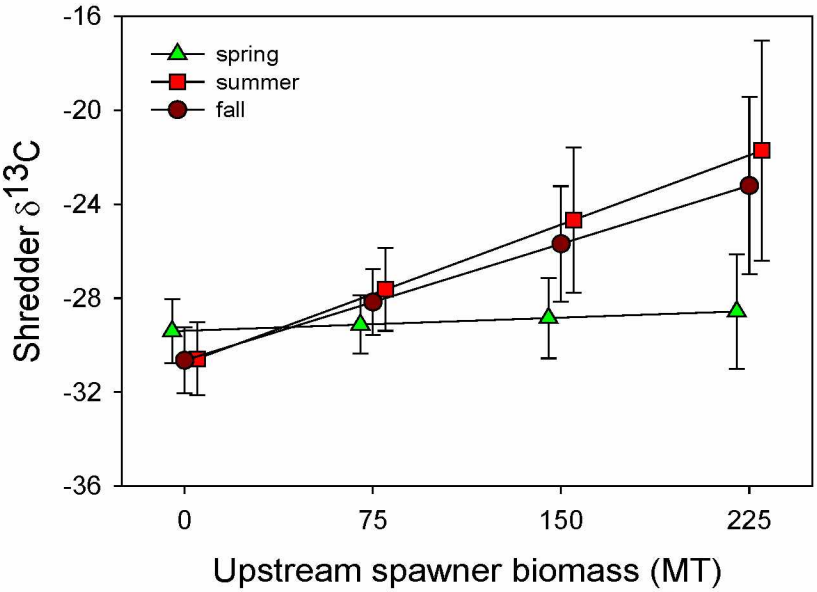

Figure 2.10. Model estimates for shredder macroinvertebrate $\delta^{13}C$ (± 95% CI) across 4 levels of upstream spawner biomass. MT=metric tons.

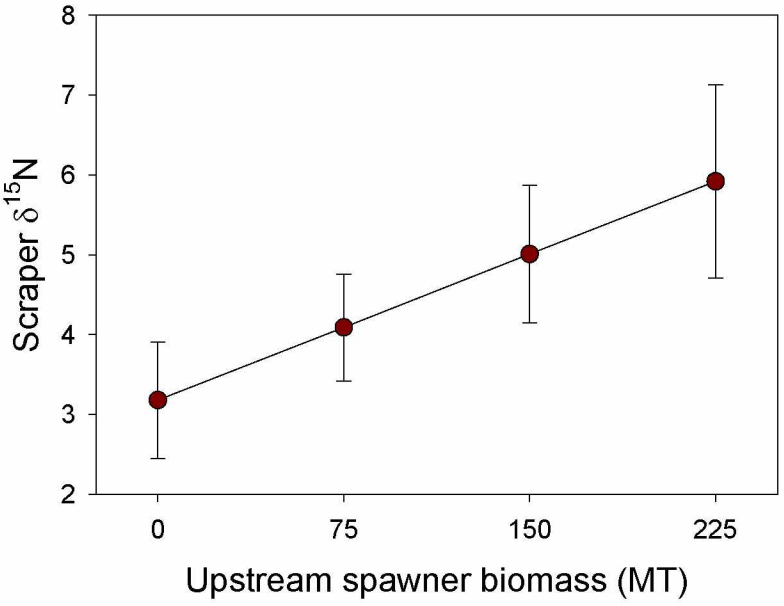

Figure 2.11. Model estimates for scraper macroinvertebrate $\delta^{15}N$ (± 95% CI) across 4 levels of upstream spawner biomass. MT=metric tons.

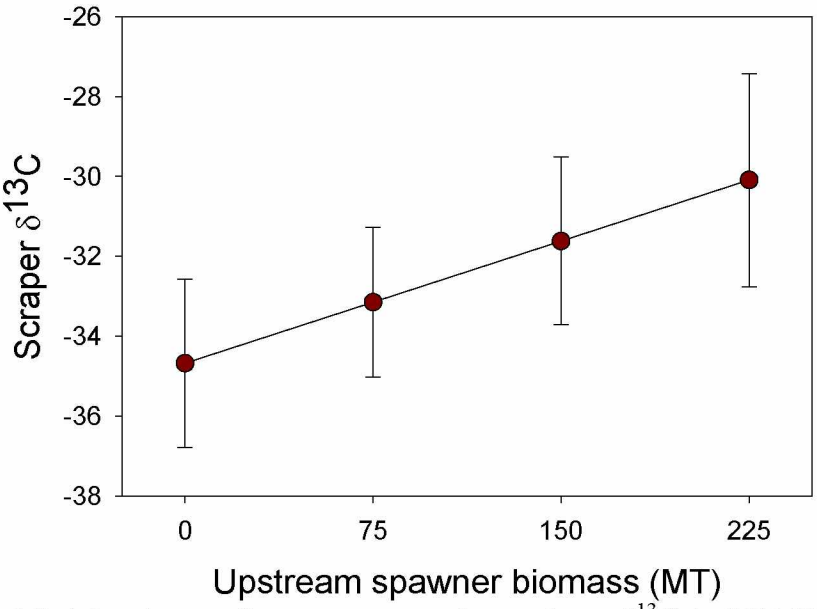

Figure 2.12. Model estimates for scraper macroinvertebrate $\delta^{13}C$ (± 95% CI) across 4 levels of upstream spawner biomass. Estimates are for spring samples in the Cooper Landing region. MT=metric tons.

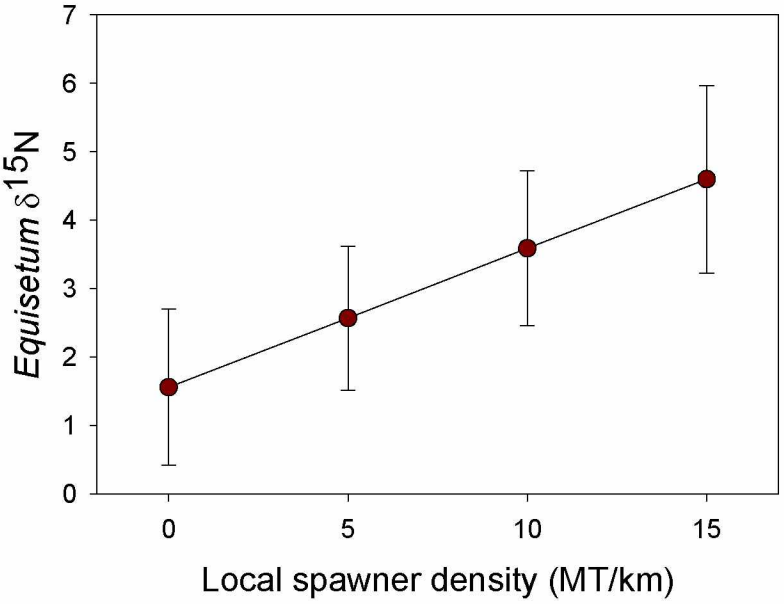

Figure 2.13. Model estimates for horsetail $\delta^{15}N$ (± 95% CI) across 4 levels of upstream spawner biomass. Estimates are for spring samples in the Cooper Landing region. MT=metric tons.

Table 2.1. Physical characteristics and estimated spawner biomass for the nine study streams.

Region	Stream	Watershed area (km^2)	Length of salmon spawning area (km)	Latitude (WGS84)	Longitude (WGS84)	2004 Spawner biomass (MT)	2005 Spawner biomass (MT)
Cooper Landing	Juneau	146	0	-149.89607	60.52034	0	0
	Quartz	322	19	-149.68517	60.50594	31	13
	Russian	166	26	-149.97489	60.47819	382	222
Homer	Happy Valley	29	0	-151.73962	59.93589	0	0
	N.F. Anchor	180	27	-151.82645	59.77662	18	21
	S.F. Anchor	373	72	-151.82893	59.77114	97	123
Seldovia	China Poot	28	0	-151.19479	59.54128	0	0
	Barabara	56	11	-151.64260	59.47533	8	22
	Humpy	28	6	-151.14339	59.65756	49	148

Table 2.2. Sampling dates for the three study regions.

	Sampling dates (2005)		
Region	Spring	Summer	Fall
Cooper Landing	6/1 – 6/16	8/20 – 9/9	10/2 – 10/14
Homer	5/12 – 5/28	8/10 – 8/23	10/7 – 10/26
Seldovia	6/22 – 7/8	9/8 – 9/21	no samples

Table 2.3. Local spawner density and upstream spawner biomass during 2004 and 2005 at sampling stations on the 9 study streams.

Region	Stream	Station	2004 Local spawner density (mt/km)	2005 Local spawner density (mt/km)	2004 Upstream spawner biomass (mt)	2005 Upstream spawner biomass (mt)
Cooper Landing	Juneau	upstream	0	0	0	0
		middle	0	0	0	0
		downstream	0	0	0	0
	Quartz	upstream	0.8	0.3	2	0.6
		middle	0.8	0.3	20	8
		downstream	2	1	31	13
	Russian	upstream	22	13	4	2
		middle	7	4	268	156
		downstream	22	13	382	222
Homer	Happy Valley	upstream	0	0	0	0
		middle	0	0	0	0
		downstream	0	0	0	0
	N.F. Anchor	upstream	0.3	0.4	0.9	1
		middle	0.3	0.4	13	15
		downstream	0.3	0.4	18	21
	S.F. Anchor	upstream	0.7	0.8	3	4
		middle	2	3	73	92
		downstream	2	3	97	123
Seldovia	China Poot	upstream	0	0	0	0
		middle	0	0	0	0
		downstream	0	0	0	0
	Barabara	upstream	0.4	1	0.8	2
		middle	0.4	1	4	11
		downstream	1	3	8	22
	Humpy	upstream	8	23	5	15
		middle	8	23	25	74
		downstream	8	23	49	148

Table 2.4. Model selection results for Dolly Varden stable isotope models within Σw_i of 0.9. Data for each dependent variable are ranked by AIC_c scores (AIC_c scores are not comparable among different dependent variables). LSD = local spawner density, USB = upstream spawner biomass, DV = Dolly Varden.

Dependent variable	Model	df	AIC_c	ΔAIC_c	w_i
Dolly Varden $\delta^{15}N$	*LSD + region + DV length*	4	182.99	0.00	0.61
	LSD + region + DV length + (DV length x LSD)	5	185.36	2.37	0.19
	LSD + region	3	187.16	4.17	0.08
	LSD + region + DV length + season	6	187.62	4.63	0.06
Dolly Varden $\delta^{13}C$	*USB + region + DV length*	4	263.79	0.00	0.32
	LSD + region + DV length	4	264.59	0.80	0.22
	USB + region + DV length + (DV length x USB)	5	265.99	2.20	0.11
	USB + region	3	265.95	2.16	0.11
	LSD + region + DV length + (DV length x LSD)	5	267.03	3.24	0.06
	USB + region + DV length + season	6	268.23	4.44	0.04
	LSD + region + DV length + season	6	269.01	5.22	0.02
	USB + region + DV length + season + (DV length x USB)	7	270.43	6.64	0.01
	LSD + region	3	269.09	5.29	0.02
Dolly Varden $\delta^{34}S$	*LSD + region + DV length + (DV length x LSD)*	5	279.33	0.00	0.33
	LSD + region + season + (season x LSD)	7	279.45	0.12	0.31
	LSD + region + DV length + season + (season x LSD)	8	281.34	2.02	0.12
	LSD + region + DV length + season + (DV length x LSD) + (season x LSD)	9	282.06	2.74	0.08
	LSD + region + DV length + season + (DV length x LSD)	7	282.80	3.47	0.06

Table 2.5. Model selection results for Dolly Varden fatty acid models within Σw_i of 0.9. Data for each dependent variable are ranked by AIC_c scores (AIC_c scores are not comparable among different dependent variables). LSD = local spawner density, USB = upstream spawner biomass, DV = Dolly Varden.

Dependent variable	Model	df	AIC_c	ΔAIC_c	w_i
Dolly Varden ω3:ω6 fatty acids	*LSD + region + DV length + (DV length x LSD)*	5	241.43	0.00	0.25
	USB + region + DV length + (DV length x USB)	5	242.27	0.84	0.16
	USB + region	3	242.31	0.88	0.16
	USB + region + DV length	4	242.41	0.98	0.15
	USB + region + season	5	244.72	3.29	0.05
	LSD + DV length + (DV length x LSD)	3	245.04	3.61	0.04
	LSD + region + DV length + season + (DV length x LSD)	7	245.16	3.73	0.04
	USB + region + DV length + season	6	245.31	3.88	0.04
	LDD + region + DV length	4	246.42	5.00	0.02
Dolly Varden Fatty acid distance from salmon flesh	*LSD + region + DV length*	4	336.73	0.00	0.32
	LSD + region	3	337.08	0.35	0.27
	LSD + region + DV length + (DV length x LSD)	5	338.82	2.09	0.11
	LSD + region + DV length + season	6	340.12	3.39	0.06
	USB + region	3	340.17	3.43	0.06
	LSD + region + season	5	340.52	3.78	0.05
	USB + region +DV length	4	340.90	4.17	0.04
	LSD + region + DV length + season + (DV length x LSD)	7	342.60	5.86	0.02

Table 2.6. Model selection results for macroinvertebrate and horsetail stable isotope models within Σw_i of 0.9. Data for each dependent variable are ranked by AIC_c scores (AIC_c scores are not comparable among different dependent variables). LSD = local spawner density, USB = upstream spawner biomass.

Dependent variable	Model	df	AIC_c	ΔAIC_c	w_i
Shredder $\delta^{15}N$	*LSD + season + region + (season x LSD)*	7	201.16	0.00	0.49
	LSD + season + region	5	201.51	0.35	0.42
Shredder $\delta^{13}C$	*USB + season + (season x USB)*	5	254.30	0.00	0.45
	USB + season + region + (season x USB)	7	255.16	0.87	0.29
	USB	1	256.60	2.31	0.14
	USB + region	3	258.22	3.92	0.06
Scraper $\delta^{15}N$	*USB + region*	3	194.18	0.00	0.67
	USB + season + region	5	196.07	1.89	0.26
Scraper $\delta^{13}C$	*USB + season + region*	5	265.56	0.00	0.52
	USB + region	3	268.11	2.55	0.15
	USB + season	3	268.42	2.86	0.12
	USB + season + region + (season x USB)	7	268.63	3.06	0.11
Horsetail $\delta^{15}N$	*LSD + season + region*	4	196.43	0.00	0.53
	LSD + season + region + (season x LSD)	5	197.00	0.57	0.40

Chapter 3

Spawning salmon and the fitness of stream-dwelling fishes: Marine-derived nutrients show saturating effects on growth and energy storage in juvenile salmonids[2]

ABSTRACT

Although numerous studies have shown positive effects of marine-derived nutrients (MDN) from Pacific salmon on freshwater fishes, the amount of MDN necessary to maximize the growth and nutritional status of freshwater fishes is unknown. Identifying salmon spawner levels above which stream-dwelling fish cease to gain physiological benefits may be a direct and appropriate measure of the capacity of fish populations to utilize MDN. We collected coho salmon (*Oncorhynchus kisutch*) parr and juvenile Dolly Varden (*Salvelinus malma*) during spring and fall from 11 streams on the Kenai Peninsula, south-central Alaska, that varied widely in salmon spawner densities (0.1 to 4.8 kg/m^2). From these samples we measured RNA-DNA ratios (an index of recent growth rates) and energy density (kJ/g dry mass) as fitness measures, in addition to nitrogen stable isotopes (δ^{15}N) as a potential low-cost proxy. RNA-DNA ratios and energy density indicated a saturation response where values increased rapidly with spawner abundance up to approximately 1 kg/m^2 and then leveled off somewhat (except for Dolly Varden during fall, which were not successfully sampled at sites with high

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[2] D.J. Rinella, M.S. Wipfli, C.A. Stricker, and R. Heintz. Spawning salmon and the fitness of stream-dwelling fishes: Marine-derived nutrients show saturating effects on growth and energy storage in juvenile salmonids. Prepared for submission to Ecological Applications.

spawner abundance). Delta ^{15}N correlated strongly with spawner abundance but showed little or no evidence of a saturation response, indicating that nitrogen isotopes do not necessarily show the same MDN dose-response patterns as more ecologically relevant metrics like growth or energy storage. In coho salmon parr, $\delta^{15}N$ correlated significantly with energy density and RNA-DNA ratios during spring and fall, suggesting nutritional benefits associated with increased MDN assimilation were realized in the fall and maintained through the winter. This study shows strong linkages between MDN availability and fitness responses in juvenile salmonids and that fitness-based saturation points can help identify target spawner densities that balance salmon harvest with the ecological benefits of MDN in stream ecosystems.

Key words

marine-derived nutrients, resource subsidy, salmon management, fresh water, coho salmon, Dolly Varden char, Alaska, stable isotopes, RNA-DNA ratios, growth, energy density

INTRODUCTION

Marine derived nutrients (MDN), delivered to fresh waters by spawning Pacific salmon (*Oncorhynchus* spp.) in the form of eggs, excreta, and carcasses, constitute an important flux of energy and nutrients to stream and riparian ecosystems. In streams with substantial salmon returns, MDN can influence multiple trophic levels. MDN inputs have been linked to increased standing stocks of biofilm (Wipfli et al. 1998, 1999; Johnston et al. 2004, Mitchell and Lamberti 2005) and macroinvertebrates (Wipfli et al. 1998, 1999, Claeson et al. 2006, Lessard and Merritt 2006, Walter et al. 2006). In stream-dwelling fish, growth rate (Bilby et al. 1996, Wipfli et al. 2003, Giannico and Hinch 2007), body condition (Bilby et al. 1998), and energy storage (Heintz et al. 2004) were increased by MDN, likely leading to increased survival of juvenile salmon (Quinn and Peterson 1996, Biro et al. 2004). With the growing understanding of the importance of MDN to the productivity of stream and riparian ecosystems, a re-evaluation of salmon escapement goals to account for the ecological demand for MDN in stream ecosystems is needed (Larkin and Slaney 1997, Michael 1998, Willson et al. 1998, Gresh et al. 2000, Gende et al. 2002, Peery et al. 2003). An important step in this process will be a better understanding of the dose-response relationship between spawner abundance and the ecological benefits of MDN (Bilby et al. 2001, Gende et al. 2002, Naiman et al. 2002, Schindler et al. 2003, Wipfli et al. 2003).

Approaches using naturally occurring stable isotopes of nitrogen have provided direct evidence for incorporation of MDN in freshwater food webs. The $\delta^{15}N$ values of adult Pacific salmon range between 10 and 15 ‰ (Welch and Parsons 1993, Satterfield

and Finney 2002) and are enriched relative to freshwater and terrestrial sources (Kline et al. 1990). The isotopic disparities between these nutrient pools offer a means for measuring the contribution of MDN to freshwater food webs (Kline et al. 1990, Bilby et al. 1996, Chaloner et al. 2002, Hicks et al. 2005, Scheuerell et al. 2007).

Mesocosm experiments conducted in southeastern Alaska have examined the dose-response relationship across multiple trophic levels. Wipfli et al. (1999) found biofilm chlorophyll *a* to increase across the range of five carcass density treatments (0, 3.2, 6.4, 9.7, 13, and 16 kg wet carcass mass/m^2), although the magnitude of the response was diminished above the lowest carcass treatment (3.2 kg/m^2). Biofilm ash-free dry mass and macroinvertebrate abundance were significantly higher in the carcass treatments relative to the control but also showed no significant additive response to carcass loading beyond the lowest treatment (Wipfli et al. 1999). They also observed similar dose-response patterns with biofilm and maroinverebrates in a natural stream, but no upper threshold was detected. An additional mesocosm experiment showed that juvenile coho salmon (*O. kisutch*) had higher carbon and nitrogen stable isotope ratios (Chaloner et al. 2002), higher growth rates (Wipfli et al. 2003), and higher lipid content (Heintz et al. 2004) in treatments with salmon carcasses (1.9, 3.7, 5.6, and 7.4 kg/m^2) relative to a control, although these effects partially leveled off at carcass loadings above the lowest treatment (1.9 kg/m^2). These experiments demonstrated that stream biota have a finite capacity to utilize MDN subsidies, particularly consumers, and that once the capacity is exceeded, the addition of more carcasses may offer little or no ecological benefit to the organisms utilizing this resource. While a saturation response is clearly

demonstrated in these mesocosm experiments, measuring dose responses in natural systems would add credence to these findings.

In an effort to identify specific saturation levels, Bilby et al. (2001) collected coho salmon parr from 26 stream reaches representing a range of spawner abundance (approximately 0 to 0.8 kg/m^2) and found that $\delta^{15}N$ leveled off at approximately 0.15 kg carcass/m^2. They proposed that the observed saturation response reflected carcass loading at or above densities which provided no additional ecological benefit to juvenile coho salmon. Further, the authors suggested that the observed saturation point approximates a salmon escapement goal for western Washington streams that additionally helps meet the ecological needs of stream-dwelling fishes. However, because the isotopic composition of fish reflects the material assimilated during periods of growth (Hesslein et al. 1993, Perga and Gerdeaux 2005), it is possible that saturation in nitrogen isotope values also indicates the spawner level at which growth rates level off, although this has never been tested.

While this approach holds promise, it must be noted that some investigations of MDN dose-response have failed to show evidence of trophic saturation with increasing MDN. Johnston et al. (1997) showed that $\delta^{15}N$ values in mayflies, stoneflies, and caddisflies increased linearly with spawner abundance despite relatively high spawner densities at some of their sites (i.e., up to approximately 2.5 salmon/m^2 or 5 kg/m^2). Likewise, Walter et al. (2006) found that the abundance of caddisfly larvae increased linearly across the observed range of salmon carcass abundance (i.e., from 0 salmon to 25% coverage of transects by salmon carcasses), although it is difficult to convert these

data to mass per m^2 for comparative purposes. Lastly, Riechert et al. (2008) found $\delta^{15}N$ of coho salmon parr to increase linearly across spawner densities ranging from approximately 250 to 2000 kg/km, although their highest densities may have been too low to elicit a saturation response. If their streams are assumed to be 20 m wide, the highest carcass abundance would be approximately 0.1 kg/m^2 or if 10 m wide then 0.2 kg/m^2; these levels are near the saturation point of 0.15 kg/m^2 reported by Bilby et al. (2001). It also seems reasonable to expect that MDN saturation levels may vary by species and abundance of consumers, local environmental conditions (e.g., water temperature, turbidity, photoperiod, ambient nutrient concentrations), and the timing of spawning runs. Further, the removal of substantial numbers of spawning salmon by bears should reduce their availability to in-stream consumers and, in turn, increase the abundance of spawners necessary to saturate the system.

Another potential problem with the isotopic approach for identifying MDN contributions and saturation responses may be that isotope values confer only limited information regarding fitness. A stream-dwelling fish could, in theory, derive all of its nitrogen from marine-derived sources and yet still be in poor nutritional condition. Additionally, situations where isotope ratios do not necessarily reflect MDN assimilation are conceivable. If fish are allocating MDN resources toward lipid storage, as opposed to protein synthesis and somatic growth, it is possible that nitrogen isotopic ratios would fail to reflect the extent MDN intake and assimilation (DeNiro and Epstein 1977, Post and Parkinson 2001). It is also possible that denitrification in the riparian soils of spawning streams can lead to overestimation of the influence of MDN on nitrogen isotope values in

stream food webs (Pinay et al. 2003) or underestimation where nitrogen fixers are abundant in riparian areas (Wipfli and Musslewhite 2004).

Since growth and energy storage in stream-dwelling fish have been linked to MDN abundance (Wipfli et al. 2003, Heintz et al. 2004), a more appropriate and direct measure of a fish population's capacity to utilize MDN may be the spawner levels above which stream-dwelling fish cease to gain these physiological benefits. Growth and energy storage are fundamental measures of an organism's fitness; increased body size (Quinn and Peterson 1996) and energy reserves (Biro et al. 2004) have been linked to higher freshwater survival for coho salmon parr. During the subsequent marine stage, larger body size can translate to higher survival to adulthood (Bilton et al. 1982, Holtby et al. 1990). In this study, we examined the relationship between salmon spawner abundance and growth rates and energy density in stream-dwelling fishes as a potential method for setting salmon escapement goals. Working with coho salmon parr and juvenile Dolly Varden char collected during spring and fall from streams with a wide range of spawning salmon abundance, we indexed recent growth rates using RNA-DNA ratios and measured somatic energy density. RNA-DNA ratios in fish muscle, reflective of recent growth rates because protein synthesis requires increased cellular RNA while DNA levels remain relatively stable, has been widely used as a growth rate measure in salmonids (Ferguson and Danzmann 1990, Wang et al. 1993, Grant 1996, MacLean et al. 2008). A fish's energy density (kJ/g wet weight) largely reflects protein and lipid content while intraspecific variation in energy density is primarily related to differences in lipid storage (Shearer 1994, Anthony et al. 2000). As such, energy density is a useful measure

of nutritional status in salmonids (Dempson et al. 2004, Heintz 2008). In addition to these measures, we tested the relationship between spawner abundance and nitrogen stable isotope ratios as a potential low-cost proxy for fitness measures. If stable isotope ratios respond to spawner abundance in a manner consistent with that of fitness measures, it would suggest a link between MDN assimilation and fitness in stream-dwelling fish while also supporting the use of stable isotopes in management applications (e.g., Bilby et al. 2001).

Our main hypotheses were that growth rate and energy density in juvenile coho salmon and Dolly Varden show saturating relationships with spawner abundance, indicating the level at which nutritional benefits cease to be gained by additional spawners, and that stable isotope ratios will also saturate with spawner abundance, but not necessarily at the same saturation level. We also expect that fitness and $\delta^{15}N$ measures will show clearer relationships with spawner abundance in the fall, just after salmon spawning, compared to spring. Finally, we expect that coho salmon will show the clearest relationship with spawner abundance since Dolly Varden exhibit high individual variation in exploiting MDN resources (Heintz 2008).

METHODS

Study area

This study was conducted in 11 streams on the Kenai Peninsula, south-central Alaska that vary widely in the abundance of spawning salmon (Figure 1, Table 1). The study area spanned three ecoregions that differ in geology, climate and vegetation

(Nowacki et al. 2001) as well as dominant salmon species. All 11 streams were wadeable and had little or no glacial influence (i.e., clear water).

Four of the study streams – Glacier, Moose, Ptarmigan, and Quartz creeks – are located in the Chugach - St. Elias Mountains ecoregion (Nowacki et al. 2001). The climate is continental with approximately 64 cm of annual precipitation (Brabets et al. 1999). Underlying geology is primarily metamorphic, volcanic, and igneous rock (Brabets et al. 1999) and, as such, these streams have relatively low ambient concentrations of primary nutrients (i.e, N and P). Juneau Creek, a nearby stream in this ecoregion where salmon runs are blocked by a waterfall, showed very low primary nutrient levels, with average total nitrogen and phosphorus concentrations of 0.1 and 0.003 mg/L, respectively (Rinella, unpublished data; samples were collected spring, summer, and fall at 3 sites). Streams in this ecoregion are typified by gently-sloped mainstem channels that drain mixed spruce/birch (*Picea* spp. and *Betula papyrifera*) forest and steep tributaries that drain rugged alpine basins (Nowacki et al. 2001). Sockeye salmon (*O. nerka*) are the dominant species of spawning salmon in these four streams and, in each case, their fry migrate downstream to rear in large lakes. Spawning occurs mainly from late July through early September. Moose, Ptarmigan and Quartz creeks are within the Kenai River basin, the Kenai Peninsula's largest watershed and Cook Inlet's largest sockeye salmon producer, which supports commercial and personal use salmon fisheries in addition to Alaska's largest sport fishery. Glacier Creek is a tributary to Tustamena Lake in the Kasilof River basin. The Kasilof River, just south of

the Kenai River, is the second largest watershed on the Kenai Peninsula and is also a major sockeye salmon producer.

Four sreams, Anchor and Ninilchik rivers and Bear and Crooked creeks, are in the Cook Inlet Basin ecoregion (Nowacki et al. 2001). Here, the climate is transitional between maritime and continental with approximately 57 cm annual precipitation (Brabets et al. 1999). Streams in this area drain extensive unconsolidated glacial deposits and proglacial lake sediments (Selkregg 1974) which results in relatively high ambient nutrient concentrations. Water samples from Happy Valley Creek, a nearby stream in this ecoregion where salmon runs are blocked by a waterfall, showed average total nitrogen and phosphorus of 0.3 and 0.04 mg/L, respectively (Rinella, unpublished data; samples were collected spring, summer, and fall at 3 sites). These streams drain a rolling landscape of white spruce (*Picea glauca*) forests that have experienced extensive mortality due to the recent spruce bark beetle (*Dendroctonus rufipennis*) outbreak (Rinella et al. 2009). Riparian vegetation is typically mixed cottonwood (*Populus* spp.) and spruce along the lower alluvial stream reaches, giving way to poorly drained soils dominated by willow (*Salix* spp.), bluejoint grass (*Calamagrostis canadensis*), and sedges (*Carex* spp.) in the upper reaches. There are no lakes in this area, but wetlands comprise about 20% of the Anchor and Ninilchik basins (Mauger 2005). The Anchor and Ninilchik rivers support runs of Chinook salmon (*O. tshawytscha*), coho salmon, and steelhead trout (*O. mykiss*), as well as anadromous and resident populations of Dolly Varden char. Bear and Crooked creeks are both tributaries in the Kasilof River basin but contain different salmon populations. Bear Creek drains into Tustamena Lake and is

used primarily by spawning sockeye salmon. Crooked Creek drains into the lower Kasilof River and is used primarily by Chinook and coho salmon, with some Chinook originating from a hatchery on the lower part of the creek. Primary spawning periods are July and August for Chinook salmon, late July through early September for sockeye salmon, and mid-September through mid-November for coho salmon.

The remaining three study streams – Humpy, Windy Left, and Windy Right creeks – are in the Gulf of Alaska Coast ecoregion (Nowacki et al. 2001). This region has a maritime climate with approximately 140 cm annual precipitation (Brabets et al. 1999). Underlying geology is primarily metamorphic, volcanic, and igneous rock (Brabets et al. 1999), with some sandstone and shale deposits along Kachemak Bay (Selkregg 1974), giving these streams relatively low ambient concentrations of primary nutrients. Water samples from China Poot Creek, a nearby stream in this ecoregion where salmon runs are blocked by a waterfall, showed average total nitrogen and phosphorus of 0.2 and 0.002 mg/L, respectively (Rinella, unpublished data; samples were collected spring, summer, and fall at 3 sites). Streams in this area are typified by short, steep watersheds that flow directly into tidewater. Vegetation is dominated by white spruce and Sitka spruce (*P. sitchensis*), giving way to dense alder (*Alnus* spp.) stands at higher elevations. Humpy, Windy Left, and Windy Right creeks receive modest runs of chum salmon (*O. keta*) that spawn in early August and sizeable runs of pink salmon (*O. gorbuscha*) that spawn during mid to late August.

Calculating spawner densities

These 11 streams where selected for study because the Alaska Department of Fish and Game (ADF&G) monitors salmon escapement on them and because they exhibit a wide range of spawner densities (Figure 1, Table 1). We used 2006 salmon spawner data as predictors for MDN measures in fish collected during the fall of that year. Since our spring sampling was conducted prior to the onset of the 2006 spawning runs, we used 2005 spawner data as predictors for these samples. We used data from several different ADF&G projects as measures of spawning salmon abundance in the various streams.

On the Anchor River, ADF&G counted spawners 4 km above saltwater using a weir coupled with a DIDSON sonar for high-flow periods that rendered the weir ineffective (Kerkvliet et al. 2008). This system operated from 13 May – 9 September in 2005 and from 15 May – 24 August in 2006. The weir and sonar were rendered inoperable by flood flows in August 2006 prior to the end of the coho salmon run. Based on the timing and strength of the early part of the coho salmon run, the total run strength was estimated to be similar to that in 2005 (Michael Booz, Alaska Department of Fish and Game, personal communication). On the Ninilchik River, ADF&G operated a weir located 9 km above saltwater from 6 May – 4 August in 2005 and 30 June – 1 August in 2006. This weir counted Chinook salmon escapement but was removed too early to count coho salmon. Lacking weir counts, coho salmon escapement was estimated from 2005 and 2006 sport fishing harvest rates (ADF&G statewide harvest survey data). Concurrent harvest and weir data from a neighboring stream of similar size (Deep Creek) showed harvest rate to average 40% of the total coho salmon run, and this figure was

applied to the Ninilchik harvest data to approximate coho salmon escapement. On Crooked Creek, ADF&G operated a weir from 3 May – 9 September in 2005 and from 21 April – 4 October in 2006. Unless otherwise specified, the above ADF&G fish count data were accessed at http://www.sf.adfg.state.ak.us/Region2/Escapement/HTML/query.cfm.

In Humpy, Windy Left, and Windy Right creeks, ADF&G personnel conducted periodic ground surveys (5 or 6 surveys per season) throughout the salmon spawning reaches. They applied a stream life factor of 17.5 days to both salmon species present (pink and chum) to estimate total spawner abundance (Hammarstrom and Ford 2008; Ted Otis, ADF&G, personal communication).

In the remaining streams ADF&G personnel counted spawning salmon with one or more ground surveys in periods of relatively low streamflow during peak spawning of the dominant sockeye salmon populations (David Westerman, ADF&G, personal communication). Moose, Ptarmigan, and Glacier creeks are relatively short, and the entire extent of salmon spawning habitat was surveyed. Quartz and Bear creeks are much longer streams and were surveyed from the stream mouth to the upper extent of any substantial salmon spawning activity. In cases where more than one survey was conducted, we used the highest spawner counts.

We calculated the total spawner biomass for each stream (for both 2005 and 2006) using the year-specific average mass for individuals of each salmon species sampled from local commercial catches (Hammarstrom and Ford 2008). We measured the approximate length of each stream used by spawning salmon from ADF&G's

interactive Fish Distribution Database (http://www.sf.adfg.state.ak.us/SARR/FishDistrib/FDD_ims.cfm) which enabled stream-specific estimates of spawner biomass densities. For Quartz and Bear creeks, however, we used the length of the actual spawner survey to express the length of stream used by spawning salmon. These estimates were coupled with estimates of average stream width throughout the spawning reach to yield estimates of MDN biomass per unit area of stream bed (i.e., kg/m^2).

The escapement estimates used in this study undoubtedly contained different levels of observational error. Weir counts, which probably provided the most accurate estimates, were used on the 3 streams with the lowest spawner densities (i.e., Anchor and Ninilchik rivers and Crooked Creek), making it unlikely that spawner abundance at the low end of the spectrum was grossly underestimated. Foot surveys, relying on discrete counts of salmon in field conditions, undoubtedly underestimated spawning abundance to a greater extent. Underestimation was probably greatest among those streams where escapement estimates did not employ multiple foot surveys with stream life factors (i.e., Moose, Ptarmigan, Glacier, Quartz, and Bear creeks). Despite these limitations, spawner densities observed during field sampling were consistently in agreement with estimates derived from ADF&G data, and the estimates appear to approximate the abundance of spawners at each site. Furthermore, given the two order-of-magnitude range in estimated spawner densities, expected levels of observational error should have little or no influence on the overall results. As a post-hoc test of this assertion, we doubled the spawner densities at the above sites where underestimation was expected to be greatest

and found negligible changes in the shape of the relationships between spawner abundance and the dependent variables.

Field sampling

We conducted field sampling in early May and again in September/October to represent the beginning and end of the growing season. During each sampling bout, we used baited minnow traps to collect up to five coho salmon parr and five juvenile Dolly Varden. Fish of both species were not necessarily collected from all streams on both dates because of the lack of availability; no coho salmon were captured in Humpy Creek, no Dolly Varden were captured in Ninilchik River, and no fish were captured from Windy Left Creek in the fall. Additionally, Dolly Varden were not successfully captured in the fall at those sites with the highest spawner abundance (i.e., Humpy, Windy Left, and Windy Right creeks; see Table 2). Fish were quickly killed by anesthetizing with an overdose of MS-222 and all samples were kept on ice in the field or on liquid nitrogen in cases where field storage time exceeded a couple hours; in the lab, samples were stored in a -70 °C ultra-cold freezer until processed. We removed a small piece of white dorsal muscle (~0.05 g) for RNA-DNA, homogenized the remaining material, and split the homogenate for stable isotope and fatty acid analyses. This work was conducted under the University of Alaska Fairbanks IACUC protocol number 06-04.

Energy density analyses

Analyses of energy density were run at the National Marine Fisheries Service laboratory in Auke Bay, AK. The energy density (kJ/g dry mass) of most fish, including all fish <3.5 g total wet mass, was determined by bomb calorimetry. Samples were dried and calorimetric analysis was performed with a Parr 1425 semi-micro bomb calorimeter. The energy density of some fish, including most of larger individuals, was determined from the calorific equivalents for lipid (36.43 kJ/g) and protein (20.10 kJ/g) (Brett 1995).

Lipid was extracted from ~0.3 g of wet sample homogenate using a modification of Folch's method outlined by Christie (1982) in a Dionex Accelerated Solvent Extractor 200 with 2:1 (v:v) chloroform:methanol. Extracts were washed successively with a 0.88% KCl solution and 1:1 (v:v) methanol:deionized water in a volume equal to 25% of the extract to remove co-extractables. Excess solvent was evaporated and percent lipid was calculated gravimetrically. References were all found to be within 10% of the expected value and duplicated samples were always within 5%.

Protein content was estimated from the total nitrogen content observed in a ~0.1 g sample of dried homogenate. Nitrogen content of the sample was measured with a LECO FP 528 Nitrogen Analyzer following the Dumas method in which the homogenate was combusted at 850 °C and the expelled nitrogen measured by thermal conductivity. Total mass of protein was estimated by multiplying the total nitrogen content by 6.25 (Jones 1931, Craig et al. 1978). The instrument was calibrated daily using ethylenediaminetetraacetic acid (EDTA), and all analyses were duplicated to ensure the coefficients of variation for estimated nitrogen content were less than 15%.

For 12 samples, energy density was estimated by both bomb calorimetry and the calorific equivalents of lipid and protein. These samples indicated that energy content estimates from calorific equivalents were highly correlated with the calorimetric estimates ($R^2 = 0.91$) but that calorific equivalents consistently underestimated energy density by approximately 2 kJ/g. All calorific estimates were adjusted accordingly.

RNA-DNA analyses

RNA-DNA analyses were run at the University of Southern Mississippi following the protocol described in Wang et al. (1993). To precipitate nucleic acids, white muscle samples were homogenized, combined with perchloric acid solution, and incubated on ice. Samples were then centrifuged, the supernatant was discarded, and the pellet was washed with ice-cold perchloric acid solution.

Total DNA of each pellet was measured using the diphenylamine procedure (Burton 1956). Perchloric acid solution and diphenylamine solution were added to the pellet and incubated for 20 hours at 25 °C. The optical density of the resulting supernatant at 600 nm was compared to that of standards prepared from salmon testes (Sigma Chemical Company) to determine DNA content.

Total RNA of each pellet was measured using a modified Schmidt-Thannhauser procedure (Munro and Fleck, 1966). The pellet was incubated in a potassium hydroxide solution for 30 minutes at 37 °C to hydrolyze the RNA. DNA and protein were precipitated by cooling the digest, adding perchloric acid solution, and incubating on ice. The sample was then centrifuged and the supernatant retained. The pellet was washed

with ice-cold perchloric acid solution, and the supernatant was pooled with that from the original sample. The optical density of the pooled supernatant at 260 nm was compared to that of standards prepared from yeast RNA (Sigma Chemical Company) to determine the RNA content.

All RNA and DNA analyses were conducted in duplicate and the two readings were averaged. Duplicate RNA readings differed by an average of 3.3% and duplicate DNA readings differed by an average of 3.6%.

Stable isotope analyses

Stable isotope analyses were performed at the US Geological Survey Stable Isotope Laboratory in Denver, CO. Prior to analysis, fish homogenate samples were oven-dried, pulverized, and weighed into tin capsules (5 x 9 mm). The nitrogen stable isotope composition was determined by continuous flow-isotope ratio mass spectrometry using a Carlo Erba NC1500 elemental analyzer interfaced to a GV Optima mass spectrometer (Fry et al. 1992). Results are reported in δ-notation as deviations in parts per thousand (‰) relative to a standard as follows:

$$\delta^{15}N = (R_{sample}/R_{standard}) - 1, \qquad \text{(Equation 3.1)}$$

where R is the isotope ratio $^{15}N/^{14}N$. Nitrogen isotopic compositions ($\delta^{15}N$) were normalized against the L-glutamic acid standards USGS 40 ($\delta^{15}N$ = -4.52‰) and USGS 41 ($\delta^{15}N$ = 47.57‰) and reported relative to the internationally accepted scale (i.e., air). Analytical error was ± 0.2 ‰.

Statistical analyses

For each sampling period, we averaged data by site and by fish species (i.e., coho salmon and Dolly Varden) so that each data point for a given dependent variable represented the average value of all fish of a given species collected for a given stream and date. Because ambient stable isotope ratios varied across the study area (D.J. Rinella, manuscript in preparation), we used the isotopic composition of scraper macroinvertebrates (i.e., primary consumer; typically the caddidfly *Glossosoma* spp. and/or the mayfly *Drunella doddsi*; D.J. Rinella, manuscript in preparation) collected above anadromous fish barriers as a salmon-free baseline for each of the three ecoregions. We calculated indices of isotopic enrichment for each fish as the ‰ nitrogen isotopic enrichment above that of the salmon-free baseline for the respective ecoregion (i.e., 2.8, 6.7, and -0.1‰ for streams in the Chugach-St. Elias Mountains, Cook Inlet, and Gulf of Alaska Coast ecoregions, respectively). A similar approach was used by Bilby et al. (2001) although they used the isotopic composition of trout living above barriers to anadromous fish as salmon-free baselines for their respective watersheds. Our approach was necessitated by the lack of salmon-free reaches in most watersheds and the lack of resident salmonids in one of our salmon-free reaches (i.e., China Poot Creek in the Gulf of Alaska Coast ecoregion). We used paired-samples *t*-tests to detect seasonal differences in energy density, RNA-DNA ratios, and $\delta^{15}N$ enrichment index for both coho salmon and Dolly Varden. To determine whether or not dependent variables showed evidence of saturation with increasing spawner abundance, taking each season separately we fit a least squares linear (i.e., no saturation) and logarithmic (i.e.,

saturation) model. For each dependent variable, we then calculated Akaike information criterion adjusted for small sample size (AIC_c) for the competing linear and logarithmic models. AIC_c is a model selection tool that allows the relative goodness of fit for competing models to be compared (Akaike 1973, Burnham and Anderson 2002), where smaller AIC_c scores translate to a better fit. The relative level of support for each model was assessed by the difference in AIC_c scores; in cases where the two models differed by 2 or more AICc (i.e., $\Delta AIC_c \geq 2$), we concluded that support for the top model over the competing model was substantial (sensu Burnham and Anderson 2002).

We conducted a second analysis with the objective of further understanding the relationship between nitrogen isotopic enrichment and fitness measures in stream-dwelling fishes. Using individual fish as data points, we applied linear regression ($\alpha = 0.05$) to test the relationship between the $\delta^{15}N$ enrichment index and RNA-DNA ratios and energy density for both seasons and both fish species. This analysis further tested the assumption that increased MDN assimilation, as measured by $\delta^{15}N$ enrichment, can serve as a proxy for fitness measures in fishes. We also used *t*-tests to compare the site-specific coefficients of variation (CV) in $\delta^{15}N$ between coho salmon and Dolly Varden for both spring and fall samples to determine if $\delta^{15}N$ enrichment was more variable in Dolly Varden.

RESULTS AND DISCUSSION

Energy density

In spring samples, energy density ranged from 19.0 to 23.9 kJ/g for coho salmon and 19.9 to 23.1 kJ/g for Dolly Varden (Table 2). In fall samples, energy density ranged from 21.0 to 25.3 kJ/g for coho salmon and from 21.3 to 26.0 kJ/g for Dolly Varden (Table 2). Energy densities were higher in the fall than in the spring for both Dolly Varden ($P = 0.02$, paired-samples *t*-test) and coho salmon ($P < 0.001$, paired-samples *t*-test) (Table 3), a seasonal pattern similar to that observed in other species of cold water fishes (Berg and Bremset 1998, Finstad et al. 2003). This seasonal pattern was likely magnified in the current study because fall samples were collected during a period where eggs and flesh from spawning salmon had been supplementing the diets of stream-dwelling fish for some time.

The relationship between energy density and spawner abundance in spring samples was best described by logarithmic functions, although the level of support was substantial for coho only ($\Delta AIC_c = 1.6$ and 3.9 for coho salmon and Dolly Varden, respectively; Table 4). Logarithmic functions explained 67% of the variation in energy density for coho salmon and 65% for Dolly Varden (Figure 2). For fall coho salmon, the data showed substantial support for a logarithmic function ($\Delta AIC_c = 7.4$; Table 4), explaining 77% of the variation in energy density (Figure 2). Fall Dolly Varden data were marginally better described by the linear function ($\Delta AIC_c = 0.5$; Table 4) and relatively little of the variation was explained ($R^2 = 0.39$; Figure 2). Despite considerable effort, Dolly Varden were not collected from sites with the highest spawner densities

during the fall sampling period; a saturating relationship may exist but we were unable to sample at large enough values of the independent variable to detect it. Aside from the fall Dolly Varden samples, our data supported a diminishing increase in energy density, where energy density increased rapidly with spawner abundance until about 1 kg/m^2 and then leveled off (Figure 2). These results indicate that energy storage in juvenile coho salmon and Dolly Varden is tightly linked to spawner abundance but that fish populations have a finite capacity to convert MDN resources into energy stores.

RNA-DNA ratios

In spring samples, RNA-DNA ratios ranged from 5.1 to 16.0 for coho salmon and 7.0 to 12.6 for Dolly Varden and in fall samples ranged from 5.2 to 9.9 for coho salmon and from 6.2 to 10.0 for Dolly Varden (Table 2). To our knowledge, the highest values observed in this study exceeded that in the published literature (i.e., ~12; Wang et al. 1993). The RNA-DNA ratios were higher in the spring than in the fall for coho salmon (P = 0.007, paired-samples t-test) but were similar between seasons for Dolly Varden (P = 0.77, paired-samples t-test) (Table 3). Water temperatures in Kenai Peninsula streams are warmer in May and June than in September and October (e.g., daily means of 9 – 16 and 0 – 7 °C, respectively; Mauger 2005), increasing the potential for fish growth during the spring (Elliott 1975, Jobling 1983) despite the opportunities for direct consumption of salmon eggs and flesh during the fall. Coho salmon parr feed actively on drifting organisms (Dolloff and Reeves 1990), and their relatively rapid spring growth suggests that food was abundant at this time. The relationship between RNA-DNA ratios and

growth rates is temperature dependent, where fish sampled from warm habitats have lower RNA-DNA ratios than fish with similar growth rates sampled from cold environments (Bulow 1987, Ferguson and Danzmann 1990). Thus the difference between spring and fall growth rates may be greater than that reflected in the RNA-DNA ratios.

The relationship between RNA-DNA ratios and spawner abundance in spring samples was best described by the logarithmic function, albeit only marginally so (ΔAIC_c = 1.9 and 1.1 for coho salmon and Dolly Varden, respectively) (Table 4, Figure 3). Spawner abundance explained more of the variation in spring RNA-DNA ratio in coho salmon (R^2 = 0.61) than in Dolly Varden (R^2 = 0.43) (Figure 3). Among fall samples, there was substantial support for a logarithmic relationship for coho salmon (ΔAIC_c = 3.0) while Dolly Varden data were described only slightly better by the linear function (ΔAIC_c = 0.2) (Table 4, Figure 3). Spawner abundance explained 61% of the variation in RNA-DNA ratio for coho salmon yet very little for Dolly Varden (6%) (Figure 3). The lack of fall Dolly Varden data from sites with the highest spawner abundance made it difficult to detect any saturation effect in RNA-DNA ratios. Aside from the fall Dolly Varden samples, our data supported a diminishing increase in growth rates, where RNA-DNA ratios increased rapidly with spawner abundance up to about 1 kg/m^2 after which the response saturated (Figure 3). As was the case for energy density, our RNA-DNA results suggest that growth rates in juvenile coho salmon and Dolly Varden are tightly linked to MDN abundance but that fish populations have a finite capacity to utilize these resources.

RNA-DNA ratios cannot be translated into growth rates without conducting calibration studies, but values from the literature can provide some insight into the growth rates attained by fish sampled in this study. For salmonids and other fishes, numerous studies have shown that muscle RNA-DNA ratios above 3 to 4 indicate actively growing fish while values <1.5 indicate zero or negative growth (Bulow 1970, Wang et al. 1993, Weber et al. 2003 and citations therein, MacLean et al. 2008). For juvenile pink salmon reared at 7.8°C, RNA-DNA ratios ranging between approximately 1.5 and 12 corresponded to growth of approximately -50 to 1800 mg over a span of 6 weeks (Wang et al. 1993). For atlantic salmon (*Salmo salar*) smolts reared at an average temperature of 10.7°C, RNA-DNA ratios ranging between approximately 2 and 8.5 corresponded to weight-based specific growth rates of approximately -0.4 to 1.5 %/day (MacLean et al. 2008). Based on these literature values, it is apparent that growth was positive for both species during spring and fall sampling, and that the highest RNA-DNA ratios observed in this study likely corresponded to high growth rates.

Delta^{15}N enrichment index

In spring samples, the coho salmon $\delta^{15}N$ enrichment index (i.e., ‰ isotopic enrichment above scraping macroinvertebrates from a salmon-free site in the same ecoregion) ranged from 3.1 to 10.7 ‰ while that for Dolly Varden $\delta^{15}N$ ranged from 3.8 to 11.5 ‰ (Table 2). In fall samples, the $\delta^{15}N$ enrichment index ranged from 2.6 to 7.6 ‰ in coho salmon and 4.0 to 7.0 ‰ in Dolly Varden (Table 2). For both fish species, the lowest values for $\delta^{15}N$ enrichment index were between 2.6 and 4.0 ‰, and these values

occurred at sites with the fewest spawning salmon (Figure 4). Since isotopic fractionation alone would account for 3 ‰ enrichment over the food source (DeNiro and Epstein 1981, Minagawa and Wada 1984), our data suggest that juvenile coho salmon and Dolly Varden assimilated little or no MDN at those sites with the fewest spawners. The $\delta^{15}N$ enrichment index was >8 ‰ at sites with the most spawners (Figure 4), indicating MDN assimilation (Kline et al. 1990, Bilby et al. 1996, Chaloner et al. 2002).

While $\delta^{15}N$ varied with spawner abundance when multiple streams were considered, there was no seasonal effect on the $\delta^{15}N$ enrichment index within specific streams. For both coho salmon and Dolly Varden, $\delta^{15}N$ from spring and fall samples did not differ significantly (paired-samples *t*-test, $P = 0.75$ and 0.41, respectively; Table 3), so we averaged the $\delta^{15}N$ values from the two seasons. Since spawner densities from 2005 and 2006 were highly correlated ($R = 0.97$), we used the average of the two estimates as the predictor for $\delta^{15}N$ data. AIC_c values indicated substantial support for a logarithmic relationship between the coho salmon $\delta^{15}N$ enrichment index and spawner abundance ($\Delta AIC_c = 0.2.3$; Table 4). Likewise, this function described a substantial amount of the variation in coho salmon $\delta^{15}N$ enrichment index ($R^2 = 0.79$; Figure 4). For the $\delta^{15}N$ enrichment index in Dolly Varden, the logarithmic function offered a marginally better approximation of the data ($\Delta AIC_c = 0.8$; Table 4), yet this function described 78% of the variation (Figure 4). Thus our data showed substantial evidence for saturation in $\delta^{15}N$ enrichment with increasing spawner abundance for coho salmon parr but little evidence for such a relationship in Dolly Varden, suggesting that saturation in $\delta^{15}N$

enrichment may serve as a reasonable proxy for saturation in fitness measures, at least for coho salmon parr.

Sequestration of marine-derived N in fish proteins offers one potential explanation for the lack of season effect on $\delta^{15}N$. Most salmon spawning in Kenai Peninsula streams occurs from June through September, coincident with relatively warm water and the highest seasonal growth potential. Stream-dwelling fishes have the potential to assimilate substantial quantities of MDN during this time and, soon afterward, water temperatures fall to 0 °C and remain there through April (Sue Mauger, Cook Inletkeeper, unpublished data; Kyle and Brabets 2001). During this period, fish metabolism slows and growth all but ceases, presumably maintaining the MDN signature throughout the cold winter months and until spring, when warming waters in spring are soon followed by renewed availability of MDN resources. Similarly, laboratory and field studies with whitefish (*Coregonus* spp.) demonstrated that muscle stable isotopes reflect summer growth year-round and that the effect of tissue turnover during fall and winter was negligible (Hesslein et al. 1993, Perga and Gerdeaux 2005).

The relationship between $\delta^{15}N$ and fitness measures

Regression analysis showed that energy density and RNA-DNA ratios of coho salmon correlated positively with the $\delta^{15}N$ enrichment index for samples collected in both spring and fall (Table 5, Figures 5 and 6). Conversely, the $\delta^{15}N$ enrichment index in Dolly Varden showed no relationship with these fitness measures in either season (Table 5, Figures 5 and 6). These differences may indicate species-specific differences in the

dominant MDN uptake pathways. The tight linkage between $\delta^{15}N$ and fitness measures in coho salmon (Figures 5 and 6) point toward direct consumption of MDN (i.e., eggs and flesh) as the dominant pathway. Thus the relationship for coho salmon appears to be straightforward: increased spawner abundance leads to increased $\delta^{15}N$ (Figure 4) and, by extension, increased growth and energy storage (Figures 5 and 6). The relationship for Dolly Varden appears to be more complex. Increased spawner abundance did lead to increased $\delta^{15}N$ (Figure 4), but increased $\delta^{15}N$ did not translate to increased growth or energy storage (Figures 5 and 6). Together, these relationships suggest that indirect uptake of MDN through food web processes may be the dominant pathway for Dolly Varden. This assertion is supported by Heintz (2008), whose study of Dolly Varden from Alaskan streams (including sites in the Anchor River basin) showed that few Dolly Varden directly consume MDN but that most appear to assimilate MDN through indirect pathways. Heintz (2008) also showed that direct MDN consumption in Dolly Varden, although sporadic, was associated with higher RNA-DNA ratios and lipid levels (Heintz 2008). In a similar fashion, rainbow trout were shown to directly consume MDN while Arctic grayling benefitted indirectly by consuming benthic invertebrates dislodged by salmon spawning activities (Scheuerell et al. 2007). In our study, site-specific coefficients of variation for $\delta^{15}N$ were significantly higher in Dolly Varden than in coho salmon in both spring and fall (Table 6), suggesting higher intraspecific variation in direct MDN consumption.

Assuming that intraspecific variation in the $\delta^{15}N$ enrichment index was primarily driven by differences in MDN assimilation (Chaloner et al. 2002), the positive

correlations between the $\delta^{15}N$ enrichment index and energy density in coho salmon indicate equally strong influence of MDN assimilation on energy storage in both spring and fall (i.e., similar slope in spring and fall; Figure 5). The influence of $\delta^{15}N$ enrichment on coho salmon RNA-DNA ratios, by contrast, varied by season. The magnitude of the response was relatively small in the fall (Figure 6), suggesting a priority for energy storage at that time. In spring, $\delta^{15}N$ enrichment had a relatively large effect on RNA-DNA ratios (Figure 6), suggesting that rapid spring growth was augmented by increased MDN assimilation.

Management and ecological implications

Our data clearly show positive relationships between MDN abundance and RNA-DNA ratios, energy density, and $\delta^{15}N$ of juvenile coho salmon and Dolly Varden in both spring and fall. Given that these fish were sampled from streams in three different ecoregions that varied widely in morphometric features, ambient nutrient levels, and species composition of spawning salmon, spawner abundance appears to be an overriding factor in determining the growth rates, energy storage, and, by extension, survival (Bilton et al. 1982, Holtby et al. 1990, Quinn and Peterson 1996, Biro et al. 2004), of stream-dwelling fishes, at least in these study streams. Furthermore, MDN effects on growth rates and energy storage were evident during spring sampling, indicating that these effects carried over through winter, an important energetic bottleneck.

In terms of potential management strategies, our data indicated that two independent fitness measures in stream-dwelling fishes, RNA-DNA ratios and energy

density, showed saturation responses with spawner abundance, supporting the concept of trophic saturation as a tool to identify spawner levels that account for the needs of stream ecosystems (Bilby et al. 2001). The effects of spawner abundance on fitness measures leveled off at approximately 1 kg/m^2. As an illustration, this translates into one sockeye salmon (averaging 2 kg; Hammarstrom and Ford 2008) for every 2 m^2 of streambed. We found evidence for a saturation response in $\delta^{15}N$ enrichment among coho, suggesting that stable isotope measures may mirror the saturation responses found in more direct and ecologically important measures of fish fitness.

Our study addressed several potential concerns raised by Bilby et al. (2001) on the application of stable isotopes as indicators of MDN saturation in stream-dwelling fish populations. Stable isotopes are not a direct measure of fish growth or fitness, making it uncertain whether $\delta^{15}N$ saturation is a valid indicator that dietary needs for a given fish population are accounted for (Bilby et al. 2001) or if nutrients other than nitrogen are accounted for (Naiman et al. 2002). Bilby et al. (2001) also note that their study did not include samples from sites where multiple species of salmon spawn (or species other than coho salmon, for that matter) or samples collected at any time except late winter. However, the results of this study are not without limitations for the same reasons indicated in Bilby et al. (2001). Specifically, the extent to which fish in this study reared in the vicinity of their capture is unknown. Also, the actual spawner densities in some of the sample reaches may not be the same as the calculated densities due to the accuracy of survey methods but, given the two order-of-magnitude range in estimated spawner

densities, we think the methods used here did a reasonable job of approximating spawner abundance.

The juvenile coho salmon from western Washington studied by Bilby et al. (2001) showed evidence for MDN saturation at about 0.15 kg salmon carcass/m^2 while our RNA-DNA and energy density data suggest a much higher saturation level for streams on the Kenai Peninsula (~1 kg/m^2). Seasonal differences in the timing of salmon runs in relation to water temperatures may explain some of the observed differences between saturation levels. In the western Washington streams studied by Bilby et al. (2001), spawning by coho salmon (the only species of spawning salmon present) typically occurred from late October through early January, when water temperatures are likely below the range for optimal growth. In Kenai Peninsula streams, most salmon spawning occurs from June through September, coincident with relatively warm water temperatures (Mauger 2005, Kyle and Brabets 2001) that fluctuate around the optima for salmonid growth (i.e., 14 °C for Arctic char, 15.5 °C for coho salmon; Jobling 1983) for much of the period. Attendant to high growth potential, these temperatures enable high rates of feeding and gastric evacuation (Elliott 1975), creating the possibility for substantially greater direct consumption of MDN resources than at cooler temperatures. Removal of spawning salmon and carcasses by bears, a phenomenon commonly observed along the study streams (see also Hilderbrand et al. 1999), undoubtedly removed substantial quantities of MDN from spawning streams and likely contributed to the elevated biomass of spawners needed to elicit a saturation response among the Alaskan fish populations. Although entirely speculative, another possible factor contributing to different MDN

saturation rates between Washington and Alaska may be differences in the abundance of stream-dwelling fishes. If Alaskan streams supported higher densities of stream-dwelling fishes, it would follow that greater quantities of MDN would be required to elicit a saturation response. Regardless of the reason for these differences in saturation levels, it is apparent that saturation levels must be identified on a region-by-region basis.

Methods based on MDN saturation points hold promise as tools for identifying spawner levels that account for the nutritional needs of stream-dwelling fishes while allowing for a harvestable excess of salmon. Such methods could be used to guide salmon escapement goals in systems where high-value sport fisheries targeting stream-dwelling species (including Chinook and coho salmon) are in conflict with commercial salmon fisheries. In terms of MDN effects on the broader freshwater and riparian ecosystem, further research should examine the assimilative capacity and saturation responses of other ecosystem components like primary producers, macroinvertebrates, and riparian plants and animals. Finally, this study indicates that the concept of 'over-escapement' should be viewed in the context of the broader ecosystem. While Ricker stock-recruit models indicate that high escapements reduce per-spawner returns for that species (Walters et al. 2004, Clark et al. 2007), high numbers of spawners may provide benefits to other freshwater and riparian species (e.g., Michael 1995). Managing escapements to meet the needs of freshwater and riparian organisms will help ensure the long term productivity of salmon populations and the ecosystems they inhabit.

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Table 3.1. Estimated spawning salmon densities of study streams in 2005 and 2006.

Study streams by ecoregion	Spawning reach length (km)	Average stream width (m)	2005 Salmon abundance (no./km)	2005 Salmon biomass (kg/m^2)	2006 Salmon abundance (no./km)	2006 Salmon biomass (kg/m^2)	Dominant salmon species
Chugach - St. Elias Mountains ecoregion							
Glacier Cr.	2	3	1938	1.3	2407	1.6	sockeye
Moose Cr.	3	3	119	0.1	324	0.2	sockeye
Ptarmigan Cr.	5	8	608	0.2	692	0.2	sockeye
Quartz Cr.	19	8	824	0.2	3542	0.9	sockeye
Cook Inlet Basin ecoregion							
Anchor R.	106	9	283	0.1	262	0.1	chinook/coho
Bear Cr.	19	5	4441	1.8	3345	1.3	sockeye
Crooked Cr.	39	8	111	0.1	137	0.1	chinook/coho
Ninilchik R.	29	8	155	0.1	184	0.1	chinook/coho
Gulf of Alaska Coast ecoregion							
Humpy Cr.	5	7	18896	4.1	10004	2.4	pink
Windy Bay Left Cr.	8	5	9043	2.7	8183	2.6	pink
Windy Bay Right Cr.	2	4	11998	4.7	8855	3.7	pink

Table 3.2. Mean values (including sample size [n] and standard deviation [SD]) for energy density, RNA-DNA ratios, and $\delta^{15}N$ enrichment index in juvenile coho and Dolly Varden collected in spring and fall from the 11 study streams.

Dependent variable	Stream	spring coho			spring Dolly Varden			fall coho			fall Dolly Varden		
		n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD
Energy density (kJ/g dry mass)	Anchor R.	5	19.0	0.4	5	21.9	1.4	5	21.9	1.3	5	21.3	0.5
	Bear Cr.	5	22.2	0.9	5	21.8	0.1	5	25.3	1.2	5	26.0	1.9
	Crooked Cr.	5	19.8	1.0	1	19.9		5	21.0	1.3	4	22.8	1.4
	Glacier Cr.	5	21.8	1.7	5	22.6	0.8	5	23.7	0.7	5	24.6	0.3
	Humpy Cr.				4	21.9	0.9						
	Moose Cr.	5	21.5	0.8	5	20.6	0.8	3	22.6	0.5	5	21.7	1.1
	Ninilchik R.	5	19.6	0.3				5	21.0	1.1			
	Ptarmigan Cr.	5	21.9	0.6	2	20.2	0.3	4	23.7	0.2	3	25.1	2.5
	Quartz Cr.	5	21.1	0.4	5	21.8	0.4	5	24.2	0.3	5	22.9	1.7
	Windy Left Cr.	5	23.9	0.3	3	22.9	0.3						
	Windy Right Cr.	3	23.6	0.5	4	23.1	1.3	5	24.7	1.0			
RNA-DNA ratio	Anchor R.	5	9.3	0.8	5	8.2	1.3	5	7.1	1.5	5	6.2	1.4
	Bear Cr.	5	13.5	1.2	5	12.1	4.2	5	8.6	2.0	5	9.9	3.6
	Crooked Cr.	4	5.1	0.6	1	7.0		5	5.2	0.5	4	10.0	3.2
	Glacier Cr.	5	10.8	1.8	5	9.0	2.1	5	9.9	0.7	5	8.8	1.5
	Humpy Cr.				4	10.0	2.8						
	Moose Cr.	5	11.2	1.5	5	10.2	2.0	3	7.3	1.5	5	8.3	2.7
	Ninilchik R.	5	5.3	1.0				5	5.8	1.2			
	Ptarmigan Cr.	5	12.8	1.3	2	7.1	1.5	4	8.5	1.8	3	9.6	2.4
	Quartz Cr.	5	9.6	1.3	5	11.2	2.0	5	6.7	1.5	5	8.6	2.8
	Windy Left Cr.	5	14.7	1.4	3	11.4	2.1						
	Windy Right Cr.	3	16.0	2.2	4	12.6	1.0	5	9.4	2.9			
$\delta^{15}N$ enrichment index (‰)	Anchor R.	5	3.5	0.5	5	3.8	0.3	5	3.5	1.7	4	4.2	1.0
	Bear Cr.	5	6.8	0.5	5	7.1	0.8	5	4.5	1.2	2	6.1	0.3
	Crooked Cr.	4	3.7	0.8				4	2.7	0.7	4	4.0	1.0
	Glacier Cr.	4	7.5	0.5	3	6.6	1.4	4	7.4	0.3	4	6.5	1.6
	Humpy Cr.				3	10.0	4.2						
	Moose Cr.	5	5.9	0.6	4	6.0	1.7	2	5.7	0.3	2	6.1	1.7
	Ninilchik R.	4	3.1	0.6				5	2.6	0.6			
	Ptarmigan Cr.	5	5.8	0.3	2	5.9	2.3				1	6.5	
	Quartz Cr.	4	4.9	0.3	4	4.1	1.0	5	7.6	0.8	2	7.0	1.2
	Windy Left Cr.	2	9.2	0.1	3	11.5	1.1						
	Windy Right Cr.	2	10.7	0.1	4	9.9	1.6						

Table 3.3. Results of paired-samples *t*-tests comparing site-specific energy density and RNA-DNA ratios in juvenile coho and Dolly Varden between spring and fall. *Denotes significant difference at $\alpha = 0.05$.

Dependent variable	Species	Season	*n*	Mean value	*P*-value
Energy density	coho	spring	9	21.2	0.0001*
		fall		23.1	
	Dolly Varden	spring	7	21.3	0.02*
		fall		23.5	
RNA-DNA ratio	coho	spring	9	10.4	0.007*
		fall		7.6	
	Dolly Varden	spring	7	9.3	0.77
		fall		9.0	

Table 3.4. Model selection results comparing linear and logarithmic relationships for energy density, RNA-DNA ratios, and $\delta^{15}N$ enrichment index in juvenile coho and Dolly Varden collected in spring and fall.

Dependent variable	Season	Species	Model	AIC_c	ΔAIC_c
Energy density	spring	coho	logarithmic	30.3	0
			linear	31.9	1.6
		Dolly Varden	logarithmic	23.7	0
			linear	27.7	3.9
	fall	coho	logarithmic	24.6	0
			linear	32.0	7.4
		Dolly Varden*	linear	28.2	0
			logarithmic	28.6	0.5
RNA-DNA ratio	spring	coho	logarithmic	48.2	0
			linear	50.1	1.9
		Dolly Varden	logarithmic	40.0	0
			linear	41.0	1.1
	fall	coho	logarithmic	29.2	0
			linear	32.2	3.0
		Dolly Varden*	linear	27.0	0
			logarithmic	27.2	0.2
$\delta^{15}N$ enrichment index	spring and fall combined	coho	logarithmic	34.0	0
			linear	36.2	2.3
		Dolly Varden	logarithmic	36.6	0
			linear	37.4	0.8

*Dolly Varden were not successfully sampled during fall at sites with high spawner abundance

Table 3.5. Results of regression analysis between $\delta^{15}N$ enrichment index and energy density and RNA-DNA ratio for juvenile coho and Dolly Varden sampled in spring and fall. *Denotes significant difference at $\alpha = 0.05$.

Dependent variable	Season	Species	*n*	*df*	*SS*	*F*	*P*-value
Energy density	spring	coho	40	1	66.2	67.7	<0.0001*
		Dolly Varden	33	1	4.7	4.0	0.053
	fall	coho	30	1	38.8	17.9	0.0002*
		Dolly Varden	18	1	4.0	1.4	0.260
RNA-DNA ratio	spring	coho	39	1	293.2	61.8	<0.0001*
		Dolly Varden	33	1	2.8	0.3	0.561
	fall	coho	30	1	27.2	8.8	0.006*
		Dolly Varden	19	1	10.6	1.2	0.289

Table 3.6. Results of *t*-tests comparing the site-specific coefficients of variation (CV) between juvenile coho and Dolly Varden sampled in spring and fall. *Denotes significant difference at $\alpha = 0.05$.

Season	Species	*n*	δ^{15}N CV	*P*- value (one-tailed)
Spring	coho	10	0.04	0.0004*
	Dolly Varden	9	0.18	
Fall	coho	7	0.06	0.003*
	Dolly Varden	5	0.13	

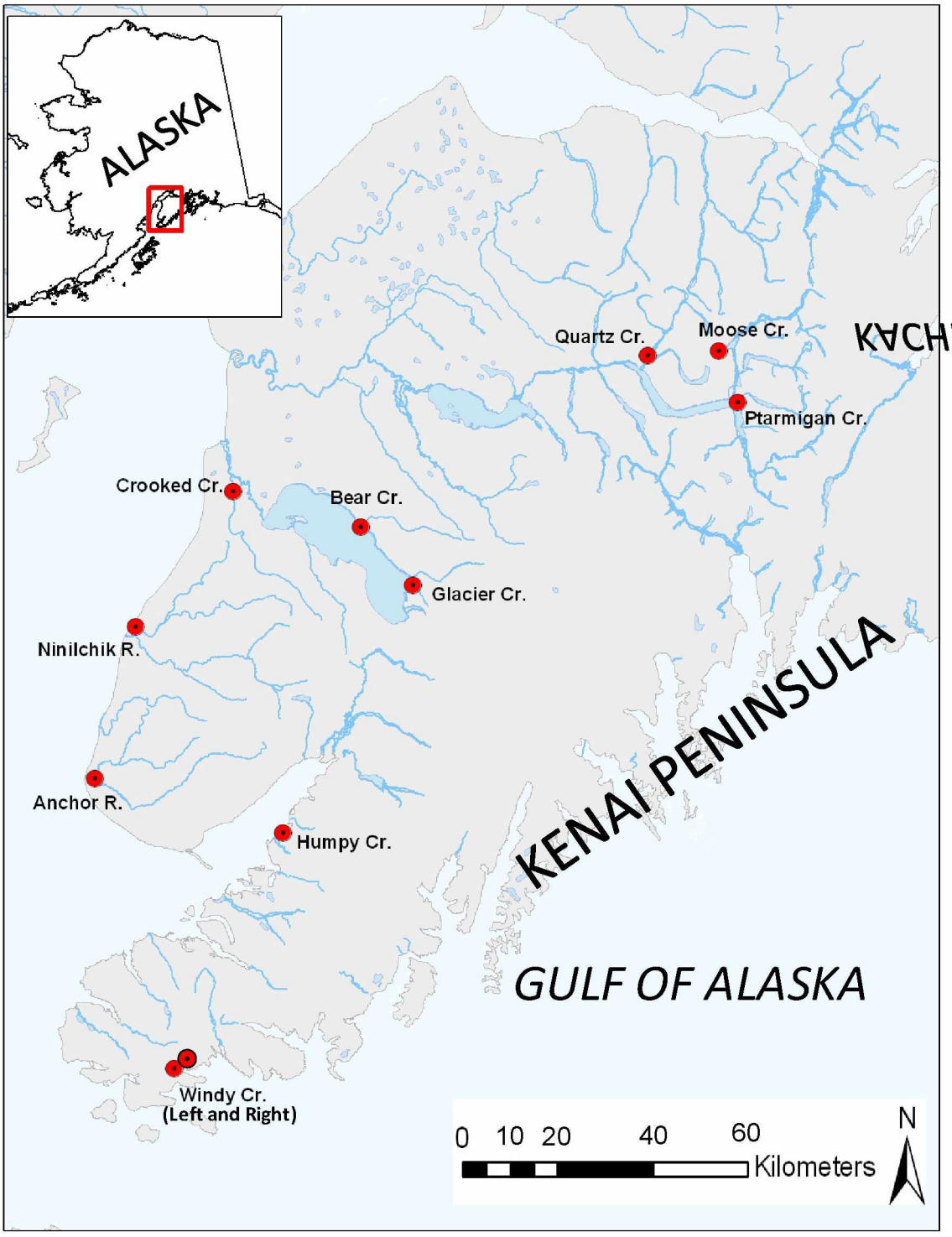

Figure 3.1. Eleven study streams on the Kenai Peninsula, Alaska.

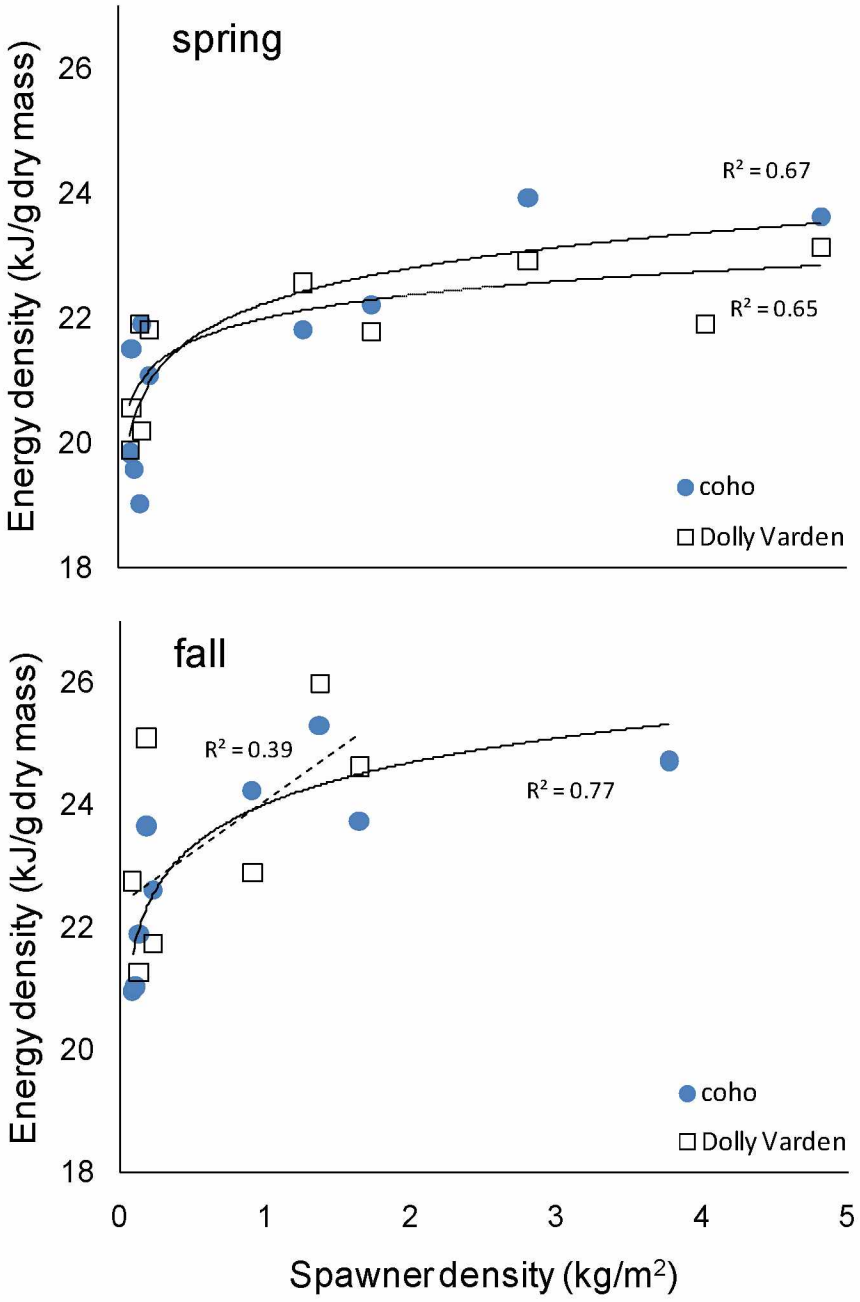

Figure 3.2. The relationship between spawning salmon abundance and energy density in juvenile coho (solid trend line) and Dolly Varden (dashed trend line) for spring and fall. Each data point represents the average of all fish of a given species (up to 5) collected at a given site.

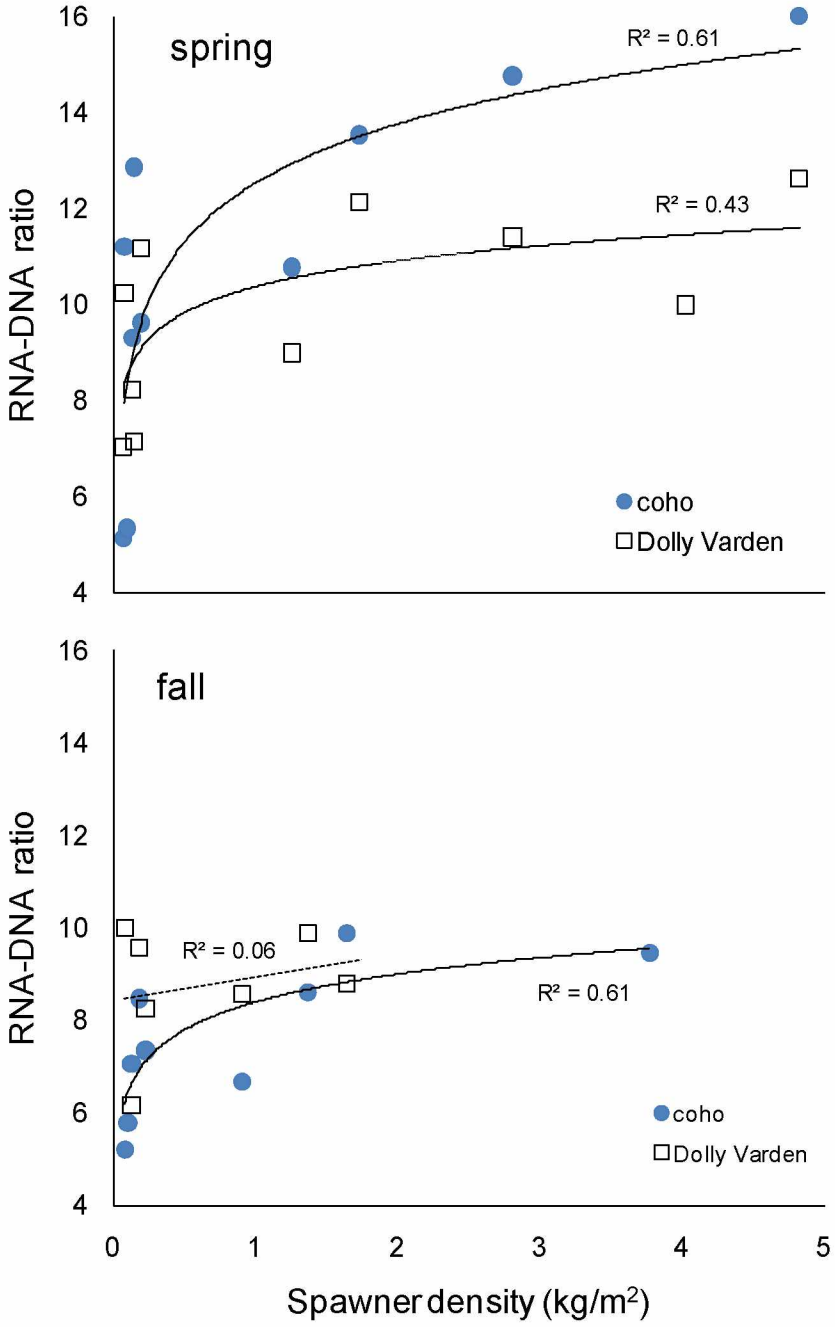

Figure 3.3. The relationship between spawning salmon abundance and RNA-DNA ratio in juvenile coho (solid trend line) and Dolly Varden (dashed trend line) for spring and fall. Each data point represents the average of all fish of a given species (up to 5) collected at a given site.

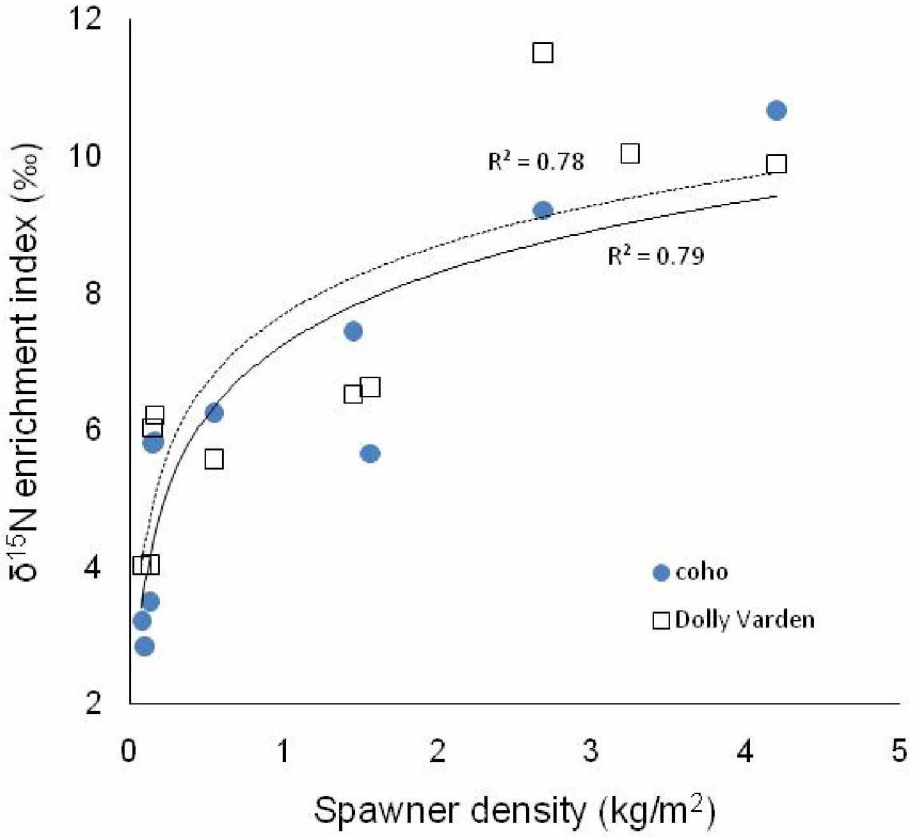

Figure 3.4. The relationship between spawning salmon abundance and $\delta^{15}N$ enrichment index for juvenile coho (solid trend line) and Dolly Varden (dashed trend line). Spring and fall data are combined. Each data point represents the average of all fish of a given species (up to 10) collected at a given site.

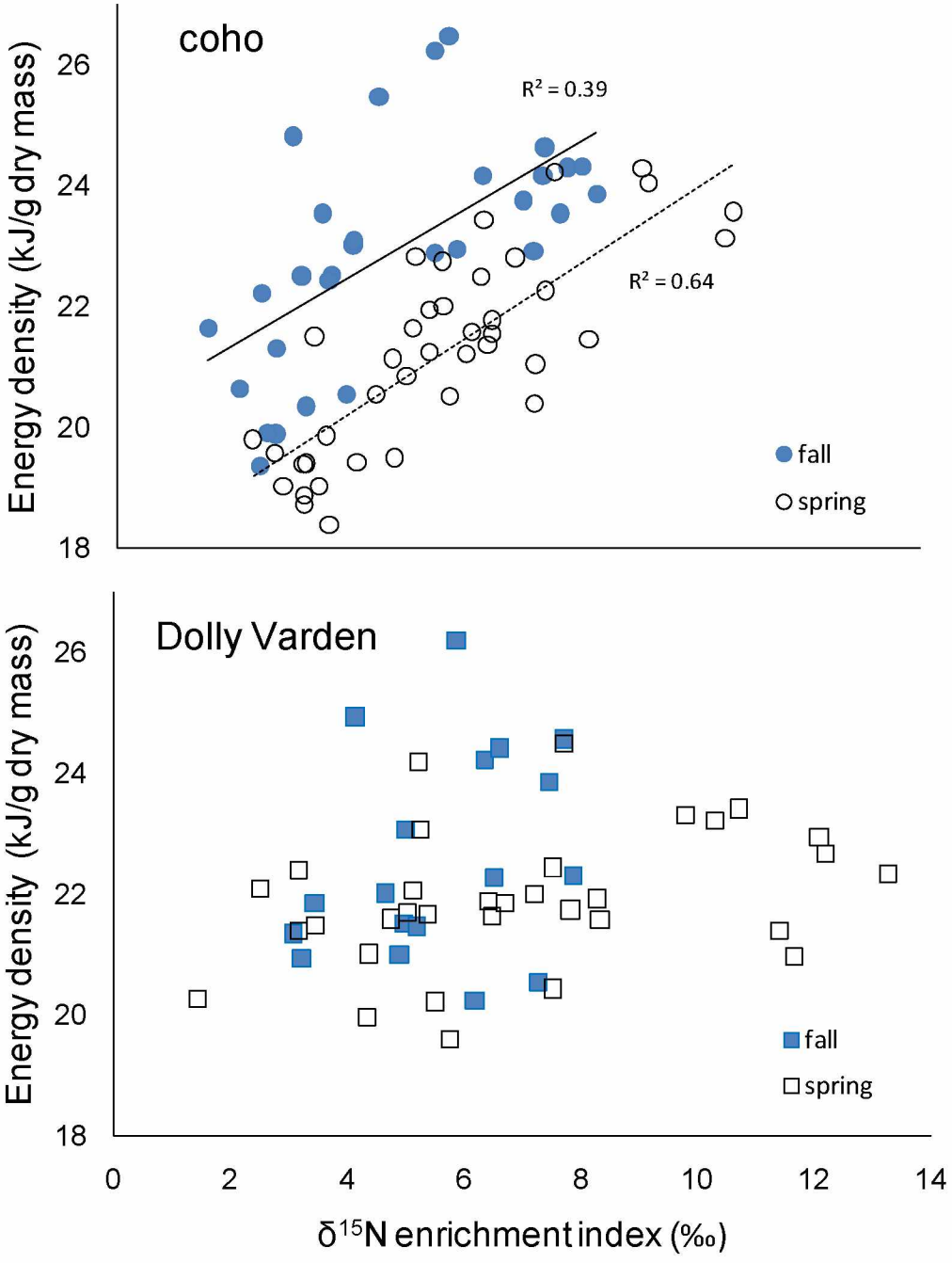

Figure 3.5. The relationship between $\delta^{15}N$ enrichment index and energy density in individual juvenile coho and Dolly Varden. Relationships are statistically significant for coho sampled in the spring (dashed trend line) and fall (solid trend line) but not for Dolly Varden.

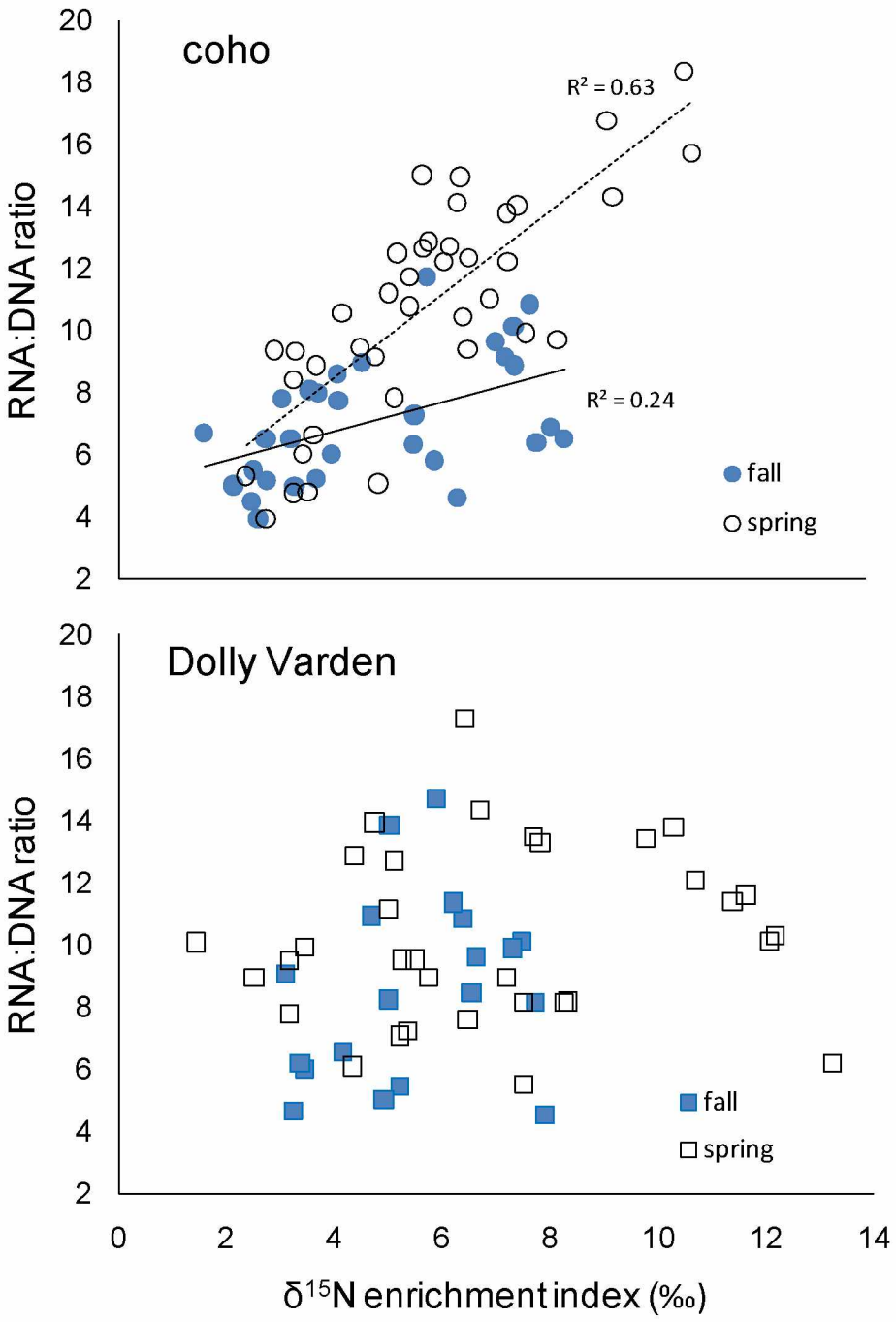

Figure 3.6. The relationship between $\delta^{15}N$ enrichment index and RNA-DNA ratio in individual juvenile coho and Dolly Varden. Relationships are statistically significant for coho sampled in the spring (dashed trend line) and fall (solid trend line) but not for Dolly Varden.

Chapter 4

Nitrogen stable isotopes in juvenile salmonids as an index of spawning salmon abundance[3]

ABSTRACT

Monitoring spawning salmon returns is crucial for the sustainable management of Pacific salmon (*Oncorhynchus* spp.) stocks, however thousands of rivers and streams, including many that contribute to subsistence, sport, and commercial fisheries, currently receive no monitoring whatsoever. A monitoring approach that is sufficiently inexpensive, easy to use, and accurate could be adopted in situations where existing techniques render monitoring too expensive or otherwise impractical. Such an approach would allow biologists to track salmon returns at many more streams than is currently feasible. We tested a quick and inexpensive method for estimating spawning Pacific salmon abundance based on a measure of marine-derived nutrient assimilation in stream-dwelling fishes (one anadromous and one resident species) on the Kenai Peninsula, south-central Alaska. We analyzed nitrogen stable isotope ratios ($\delta^{15}N$) in coho salmon parr and Dolly Varden from streams with a wide range of spawner abundance (0 – 28 metric tons [MT] spawner biomass/km), developed models that estimated spawner abundance from $\delta^{15}N$ and covariates, and compared the model estimates to traditional escapement estimates. Jackknife estimates from coho salmon parr were tightly correlated

[3] D.J. Rinella and M.S. Wipfli. Nitrogen stable isotopes in juvenile salmonids as an index of spawning salmon abundance. Prepared for submission to North American Journal of Fisheries Management.

with observed values (R = 0.95, n = 17) and had a mean absolute deviation of 1.4 MT spawner biomass/km. As hypothesized, jackknife estimates made with Dolly Varden correlated less precisely with observed values (R = 0.82, n = 49) and had a higher mean absolute deviation (2.7 MT spawner biomass/km). The slope of regression lines forced through the origin were not significantly different than 1 for either species, indicating that the models provided unbiased estimates of spawner abundance. Our results indicate that the isotopic method does a good job of estimating spawner abundance, especially when coho salmon parr are used. Application of this method has the potential to substantially increase the number of individual fish stocks that can be monitored annually since the required field effort is minimal and analytical expenses are low.

INTRODUCTION

Maintaining adequate spawning populations for individual stocks is a fundamental requirement of sustainable fisheries management (Knudsen et al. 2000). However, routine escapement monitoring occurs in only a small fraction of the water bodies where Pacific salmon (*Oncorhynchus* spp.) spawn, and thousands of streams, including many that contribute to subsistence, sport, and commercial fisheries, are not currently monitored.

Many different techniques are currently used and all have drawbacks. Census techniques such as weirs, video monitoring, and sonar are expensive in terms of labor and/or equipment, which prohibits their widespread application. Survey techniques, whether by air or on foot, are subject to observer bias and compromised by adverse

viewing conditions. Additionally, each of these techniques require that field data be collected only during well-defined, and sometimes unpredictable, timeframes (e.g., during riverine migration or spawning). The development of quick, inexpensive, and flexible methods would permit salmon stock monitoring in many streams where existing techniques cannot practically be applied.

One method that holds potential is based on the nitrogen stable isotope ratios ($\delta^{15}N$) of fishes that live in streams where salmon spawn. Stream-dwelling fishes typically consume substantial quantities of salmon eggs, the flesh of salmon that have spawned and died, and aquatic invertebrates that have fed upon rotting salmon. These marine-derived nutrients (MDN) are incorporated into the bodies of stream-dwelling fishes and, because they have characteristic nitrogen isotopic properties, stable isotope analysis can be used to quantify the contribution of MDN to the diets of stream-dwelling fishes (Kline et al. 1990, Bilby et al. 1996). Several studies have shown that $\delta^{15}N$ (i.e., the assimilation of MDN) in juvenile salmonids increases with increasing spawning salmon abundances (Bilby et al. 2001, Chaloner et al. 2002, Reichert et al. 2008, Chapters 2 and 3 of this thesis), so $\delta^{15}N$ may serve as a useful estimator of salmon escapement (Reichert et al. 2008).

Stable isotopes reflect the assimilation of MDN because nitrogen, an important and often limiting nutrient, occurs as two forms (^{14}N and ^{15}N) and the nitrogen in spawning salmon has levels of ^{15}N that are much higher than the rest of the freshwater nitrogen pool. Specifically, the nitrogen isotopic composition of adult Pacific salmon ranges from 10 to 15 ‰ (Welch and Parsons 1993, Satterfield and Finney 2002) while

other available sources of nitrogen in rivers and streams tend to be closer to that of atmospheric nitrogen (i.e., 0‰ by convention). The isotopic disparities between these nutrient pools offer a means for directly measuring the assimilation of MDN by freshwater organisms, where isotopic enrichment corresponds to increased MDN assimilation (Kline et al. 1990, Bilby et al. 1996, Chaloner et al. 2002, Hicks et al. 2005, Scheuerell et al. 2007).

Data suggest that fishes integrate MDN signals better than other aquatic or riparian organisms. Chapter 2 of this thesis compared the effect of spawner abundance on several isotopic and fatty acid measures of MDN assimilation in shredder and grazer macroinvertebrates, riparian horsetail (*Equisetum* spp.), and juvenile Dolly Varden char (*Salvelinus malma*). Of all the response variables, nitrogen isotopes in Dolly Varden showed the clearest relationship with spawner abundance (i.e., high precision and effect size, no complicating interactions). We expect that fishes, owing to their relatively high mobility, are very adept at foraging for MDN resources, thereby ensuring the presence of MDN signals and reducing their variability among conspecifics. In addition, low intraspecific variability allows the use of relatively small sample sizes of stream-dwelling fish to characterize isotope ratios for a given location. In previous studies, the within-stream coefficient of variation (CV) for coho salmon averaged 6% while Dolly Varden were somewhat more variable, with a CV of 13% (Chapter 3 of this thesis). Likewise, Bilby et al. (2001) found CVs to exceed 10% at only three of 23 sites for coho salmon (*Oncorhynchus kisutch*) parr and only two of 23 sites for cutthroat trout (*O. clarki*), leading them to conclude that a relatively small sample (three fish per species per stream)

offered a reasonable representation. We expected that juvenile coho salmon would be better indicators of salmon abundance than Dolly Varden because there appears to be much less intraspecific variation in MDN consumption among coho salmon parr than among juvenile Dolly Varden (Heintz 2008, Chapter 3 of this thesis).

In this paper we develop and test models that estimate local spawner abundance for a stream reach (on a biomass per-unit-area basis) based on the isotopic composition of stream-dwelling fishes captured there. This paper represents, to our knowledge, the first attempt to infer spawner abundance from MDN signals in freshwater biota and the first attempt to validate such predictive models. This method has the potential to substantially increase the number of individual fish stocks that can monitored annually since the required field effort is minimal (i.e., sampling ~five coho salmon parr) and analytical expenses are low (~$5 per fish). The method could be applied at many streams where escapement is currently not monitored, including those that host subsistence, personal use, and/or sport fisheries and those that contribute to mixed-stock commercial fisheries.

Our approach was to develop models that use $\delta^{15}N$ in juvenile stream-dwelling fishes to estimate spawner abundance. To assess model accuracy, we compared estimates from the models to those derived using traditional spawner monitoring methods.

METHODS

Study sites

This study was conducted at 31 sites on 17 streams on the Kenai Peninsula, south-central Alaska. These streams were chosen because the Alaska Department of Fish and

Game tracks spawner escapement on them and they vary widely in the abundance of spawning salmon (Figure 1, Table 1). The study area spanned three ecoregions that differ in geology, climate and vegetation (Nowacki et al. 2001) as well as dominant salmon species. All 11 streams were wadeable and had little or no glacial influence (i.e., they had clear water).

Six of the study streams – Glacier, Juneau, Moose, Ptarmigan, and Quartz creeks and the Russian River – are located in the Chugach - St. Elias Mountains ecoregion (Nowacki et al. 2001). The climate is continental with approximately 64 cm of annual precipitation (Brabets et al. 1999). Streams in this ecoregion are typified by gently-sloped mainstem channels that drain mixed spruce/birch (*Picea* spp. and *Betula papyrifera*) forest and steep tributaries that drain rugged alpine basins (Nowacki et al. 2001). Sockeye salmon (*O. nerka*) are the dominant species of spawning salmon in these streams (with the exception of Juneau Creek where salmon runs are blocked by a waterfall near the mouth), and their fry migrate to rear in large lakes. Spawning occurs mainly from late July through early September.

Five of the study streams are in the Cook Inlet Basin ecoregion (Nowacki et al. 2001): Anchor and Ninilchik rivers and Bear, Crooked, and Happy Valley creeks. Here, the climate is transitional between maritime and continental with approximately 57 cm annual precipitation (Brabets et al. 1999). These streams drain a rolling landscape of white spruce (*P. glauca*) forests that have experienced extensive mortality due to a recent spruce bark beetle (*Dendroctonus rufipennis*) outbreak (Rinella et al. 2009). Soils in the upper reaches are poorly drained and dominated by willow (*Salix* spp.), bluejoint grass

(*Calamagrostis canadensis*), and sedges (*Carex* spp.); wetlands are abundant (i.e., comprising about 20% of the Anchor and Ninilchik basins; Mauger 2005). The Anchor and Ninilchik rivers and Crooked Creek support runs of Chinook salmon (*O. tshawytscha*) and coho salmon, and some of the Crooked Creek Chinook originate from a hatchery on the lower part of the creek. Bear Creek is used primarily by spawning sockeye salmon. Happy Valley Creek receives no salmon runs due to a waterfall just above tidewater. Primary spawning periods are July and August for Chinook salmon, late July through early September for sockeye salmon, and mid-September through mid-November for coho salmon.

The remaining four study streams – Humpy, Barabara, Windy Left, and Windy Right creeks – are in the Gulf of Alaska Coast ecoregion (Nowacki et al. 2001). This region has a maritime climate with approximately 140 cm annual precipitation (Brabets et al. 1999). Streams in this area are typified by short, steep watersheds that flow directly into tidewater. Vegetation is dominated by white spruce and Sitka spruce (*P. sitchensis*), giving way to dense alder (*Alnus* spp.) stands at higher elevations. These streams receive modest runs of chum salmon (*O. keta*) that spawn in early August and sizeable runs of pink salmon (*O. gorbuscha*) that spawn during mid to late August.

Field sampling

We collected fish samples for stable isotope analysis in the spring and fall of 2005 and 2006. Sampling periods were 12 May – 8 July and 2 October – 26 October in 2005 and 1 May – 28 June and 20 September – 30 October in 2006. We collected spring

samples prior to the onset of salmon spawning in their respective streams and fall samples following major spawning activity in their respective streams. Since isotopic signals associated with MDN assimilation appear to be maintained throughout the winter season (Chapters 2 and 3 of this thesis), we expected the spring samples to reflect MDN assimilated during the previous year's spawning run and the fall samples to represent MDN assimilated during the spawning run that immediately preceded. During each sampling bout, we used baited minnow traps to collect coho salmon parr and/or juvenile Dolly Varden from each site (typically 3 – 5 individuals per species per site; Table 2). In 2005 we targeted only Dolly Varden, and in 2006 we targeted both fish species; however, targeted species were not readily captured at all sites on all dates. We quickly killed all fish by anesthetizing them with an overdose of MS-222® and kept them on ice in the field or on liquid nitrogen in cases where field storage time exceeded a couple hours; fish were stored in the laboratory at -70 °C until processed. We homogenized fish with a Tissuemizer, oven dried a subsample for 48 hours at 65 °C, then pulverized the subsample. This work was conducted under the University of Alaska Fairbanks IACUC protocol number 06-04.

Stable isotope analyses

Stable isotope analyses were performed at the US Geological Survey Stable Isotope Laboratory in Denver, CO. Homogenized samples were weighed into tin capsules (5 x 9 mm) and the nitrogen stable isotope composition determined by continuous flow-isotope ratio mass spectrometry using a Carlo Erba NC1500 elemental

analyzer interfaced to a GV Optima mass spectrometer (Fry et al. 1992). Results are reported in δ-notation as deviations in parts per thousand (‰) relative to a standard as follows:

$$\delta^{15}N = (R_{sample}/R_{standard}) - 1, \quad \text{(Equation 4.1)}$$

where R is the isotope ratio $^{15}N/^{14}N$. Nitrogen isotopic compositions ($\delta^{15}N$) were normalized against the standards USGS 40 ($\delta^{15}N$ = -4.52‰) and USGS 41 ($\delta^{15}N$ = 47.57‰) and reported relative to the internationally accepted scale (i.e., air). Analytical error was ± 0.2 ‰.

Estimated spawner abundance

We used linear regression models that relate $\delta^{15}N$ in stream-dwelling fish to the biomass of spawning salmon at respective sites during the preceding spawning season. For each sampling period, we averaged data by site and by fish species (i.e., coho salmon parr and Dolly Varden) so that each data point represented the average $\delta^{15}N$ of all fish of a given species collected for a given stream and date. Because ambient stable isotope ratios varied across the study area (see Chapter 3 of this thesis), we used the isotopic composition of scraper macroinvertebrates (i.e., primary consumer; typically the caddidfly *Glossosoma* spp. and/or the mayfly *Drunella doddsi*; D.J. Rinella, unpublished) collected above anadromous fish barriers as a salmon-free baseline for each of the 3 ecoregions. We calculated an index of isotopic enrichment for each fish as the ‰ nitrogen isotopic enrichment above that of the salmon-free baseline for the respective ecoregion (i.e., 2.8, 6.7, and -0.1‰ for streams in the Chugach-St. Elias Mountains, Cook

Inlet, and Gulf of Alaska Coast ecoregions, respectively). A similar approach was used by Bilby et al. (2001) although they used the isotopic composition of trout living above barriers to anadromous fish as salmon-free baselines for their watersheds. Our approach was necessitated by the lack of salmon-free reaches in most watersheds and the lack of resident salmonids in one of our salmon-free reaches (i.e., China Poot Creek in the Gulf of Alaska Coast ecoregion). We used ecoregion as a second, categorical predictor variable to account for any regional effects above and beyond differences in ambient $\delta^{15}N$. From these two predictors we developed three candidate models to estimate spawner abundance: one that used $\delta^{15}N$ alone, one that used ecoregion alone, and one that combined the two.

For each fish species, we used Akaike Information Criterion (adjusted for small sample size; AIC_c) and, in turn, Akaike weights (w_i) (Akaike 1973, Burnham and Anderson 2002) to identify the best approximating model from the model set. AIC_c is a model selection tool that compares the fit of competing models (Akaike 1973, Burnham and Anderson 2002). Values for w_i are based on the difference between AIC_c values and are interpreted as the relative likelihood that a given model best approximates the data (Burnham and Anderson 2002). By definition, the w_i values for a model set (in this case three models) sum to 1. For each fish species, we used the best approximating model (i.e., the one with the highest w_i) to estimate spawner abundances.

We made estimates about two different spawning runs: fish sampled in the spring were used to estimate the prior year's spawner counts while fish sampled in the fall were used to estimate the current year's spawner counts. We assessed the predictive accuracy

of the models by jackknifing, using Dolly Varden and coho salmon data separately. Using the jackknife-estimated values, we then averaged the magnitude by which isotope-based jackknife estimates deviated from their respective observed spawner densities. This statistic, known as the mean absolute deviation (MAD), is presented here because it offers an intuitive and biologically meaningful interpretation. However, it must be noted that mean squared error (MSE) is a more appropriate statistic since linear regression parameters are conventionally set to minimize MSE and that the models would estimate even more accurately if MAD had been the fitting criteria instead of MSE. We also plotted the jackknife-estimated values against the observed spawner abundance with the regression line forced through the origin; to assess bias we examined 95% confidence intervals to determine if the slope of the line was significantly different than 1.

Observed spawner abundance

We adapted data from several ADF&G projects as measures of spawning salmon abundance for the 2004 – 2006 seasons in our salmon-bearing study streams. On the Russian, Anchor and Ninilchik rivers and Crooked Creek, ADF&G operated fish counting weirs, with the addition of a DIDSON sonar on the Anchor River (Kerkvliet et al. 2008) for periods of high flow that rendered the weir inoperable. ADF&G operated an additional weir on the North Fork of the Anchor River during 2004 (Kerkvliet et al. 2008), giving counts for the North Fork and, by subtraction, the South Fork. Since the North Fork weir was not operational in 2005, we assumed salmon species divided themselves between the forks in the same proportion as in 2004. Coho salmon counts for

the Anchor River were incomplete in 2006 due to flooding, so total escapement was assumed to be the same as in 2005 based on early run timing and strength (Michael Booz, Alaska Department of Fish and Game, personal communication). The Ninilchik River weir was removed too early to count coho salmon, so escapement was estimated based on sport fishing harvest rates (Michael Booz, Alaska Department of Fish and Game, personal communication). Unless otherwise specified, the above ADF&G fish count data were accessed at http://www.sf.adfg.state.ak.us/Region2/Escapement/HTML/query.cfm.

In Humpy, Barabara, Windy Left, and Windy Right creeks, ADF&G personnel conducted periodic ground surveys (5 or 6 surveys per season) throughout the salmon spawning reaches. They applied a stream life factor of 17.5 days to both salmon species present (pink and chum salmon) to estimate total spawner abundance (Hammarstrom and Ford 2008; Ted Otis, ADF&G, personal communication). In the remaining streams ADF&G personnel counted spawning salmon with one or more ground surveys in periods of relatively low streamflow during peak spawning of the dominant sockeye salmon populations (David Westerman, ADF&G, personal communication). Moose, Ptarmigan, and Glacier creeks are relatively short, and the entire extent of salmon spawning habitat was surveyed. Quartz and Bear creeks are much longer streams and were surveyed from the stream mouth to the upper extent of significant salmon spawning. In cases where more than one survey was conducted, we used the highest spawner counts.

We calculated the total spawner biomass for each stream (for 2004, 2005, and 2006) using the year-specific average mass for individuals of each salmon species sampled from local commercial catches (Hammarstrom and Ford 2008). The single

exception was Anchor River Chinook salmon, for which we used individual mass data from the Anchor River weir (Kerkvliet et al. 2008). We measured the approximate length of each stream used by spawning salmon from ADF&G's interactive Fish Distribution Database (http://www.sf.adfg.state.ak.us/SARR/FishDistrib/FDD_ims.cfm) which enabled stream-specific estimates of spawner densities (MT/km). During the 2005 field season we sampled three different sites on each of our study streams. For these sites, we adjusted the local spawner biomass based on ground surveys conducted over a 500-m stream reach on several dates throughout the field season.

Our estimates of spawner abundance undoubtedly contain error but, given the two order-of-magnitude range of spawner abundance found across these sites, we think these methods to a reasonable job of approximating spawner abundance and alleviated the need for extensive spawner surveys that were outside the budget of this project. Our primary objective was to prove the concept of spawner estimates using stable isotopes rather than to draw rigorous inferences.

RESULTS

For coho salmon parr, the $\delta^{15}N$ enrichment index combined with ecoregion (w_i = 0.70) better approximated the data than either $\delta^{15}N$ index (w_i = 0.29) or ecoregion (w_i = 0.002) alone. As such, we used the following model:

$$y_i = \beta_0 + \beta_c x_{ci} + \beta_g x_{gi} + \beta_n n_i + e_i. \quad \text{(Equation 4.2)}$$

where y_i is spawner biomass in stream i. The parameter β_0 is the mean LSD (local spawner density; MT/km) for the Chugach - St. Elias Mountains ecoregion when n_i

equals zero. The difference between β_0 and the mean LSD for the Cook Inlet Basin ecoregion at a given n_i level is given by β_c. The difference between β_0 and the mean LSD for the Gulf of Alaska Coast ecoregion at a given n_i level is given by β_g. Elements of the x vectors equal 1 for the ecoregion being estimated and zero for all other ecoregions. The parameter β_n describes the effect of mean $\delta^{15}N$ for a given site, and e_i is normally distributed random error with a mean of zero.

Jackknife estimations of spawner abundance made using coho salmon parr were tightly correlated with observed values (R = 0.95, n = 17; Figure 2). On average, estimates made using coho salmon parr deviated from the observed spawner densities by 1.4 MT/km (i.e., the MAD). The slope of a regression line forced through the origin was 0.99 and its 95% confidence interval is nearly centered on 1 (95% CI = 0.85, 1.12), indicating that the model provides unbiased estimates of spawner biomass.

For Dolly Varden, the model that combined $\delta^{15}N$ enrichment index and ecoregion (w_i = 0.55) better approximated the data than either $\delta^{15}N$ index (w_i = 0.46) or ecoregion (w_i < 0.001) alone. As such, our Dolly Varden model had the same form as Equation 1, except that we natural log transformed the dependent variable to better meet regression assumptions.

Jackknife estimations made using Dolly Varden also correlated with observed values (R = 0.82, n = 49; Figure 3), although not as tightly as those made with coho salmon parr. Because of the natural log transformation, we exponentiated spawner abundances prior to calculating MAD to simplify interpretation of the statistic. Thus, the MAD of estimates made with Dolly Varden was 2.7 MT/km, nearly twice that of

estimates made with coho salmon parr. The slope of a regression line forced through the origin was 0.98, and its 95% confidence interval is nearly centered on 1 (95% CI = 0.86, 1.11), indicating a lack of bias.

DISCUSSION

Our results showed that spawner estimates derived from stable isotopes in stream-dwelling fishes were predictive of those derived by traditional census and survey methods, especially when isotope-based estimates were made using coho salmon parr. The coho salmon parr MAD translates to roughly 900 pink salmon or 220 Chinook salmon per stream km while the Dolly Varden MAD translates to almost twice that number. These levels of error may render our method unacceptable for comparing among or within stream reaches that receive low densities of spawning salmon, but our method may prove adequate for detecting large differences among systems and large year-to-year differences within systems (e.g., many pink and sockeye salmon systems). It should also be noted that some amount of the discrepancy between estimated and observed values stemmed from error in the traditional spawner estimates. Given their sporadic and variable utilization of MDN resources (Heintz 2008, Chapter 3 of this thesis), the data confirmed our hypothesis that Dolly Varden would be the poorer indicator of spawner abundance.

To assess the extent to which the predictive power of these models was strengthened by the stable isotope data, we omitted the $\delta^{15}N$ term and made jackknife estimates using only the ecoregion terms. Ecoregion alone turned out to be a relatively

poor estimator of spawner abundance. Using ecoregion alone, the MAD for coho salmon parr was 3.0 MT/km, an error rate more than double the model that included $\delta^{15}N$. The MAD for Dolly Varden using ecoregion alone was 3.8 MT/km, 40% higher than the model that included $\delta^{15}N$. As such, the inclusion of $\delta^{15}N$ data substantially improved the performance of both models.

Application of this method will not provide data on in-season run strength, run timing, or the relative contributions of different salmon species, so it has no potential to supplant traditional survey and census method for making in-season management decisions. However, it could offer an inexpensive opportunity to collect a second, independent index of salmon escapement on streams where the primary monitoring tool (e.g., aerial survey) is useful for in-season management but may not provide estimates of total spawning escapement. Other advantages are that samples could be collected by non-biologists (volunteers, interns, etc.) without the need for extensive training and that, unlike visual surveys, field data collection is not subject to observer bias or adverse viewing conditions (i.e., high water). One final advantage is that sampling need not be timed to coincide with spawning runs. Prior work has shown that isotopic signals associated with MDN assimilation appear to be maintained throughout the winter and into the spring (Chapters 2 and 3 of this thesis), creating a broad window for field sampling.

We anticipate that the method could be applied in two different ways. In the simplest approach, the method could be used to index year-to-year variation spawner abundance at selected site(s) within spawning streams of interest. Here, spawner

abundance at index sites is assumed to be representative of the stream's overall escapement. In a more complex approach, the method could be used to infer the total spawning escapement of a stream. This latter mode will require some assumptions or some data about the distribution of spawners within the stream and is probably best suited for use in small drainages.

The use of this isotopic approach is contingent upon a non-saturating relationship between $\delta^{15}N$ enrichment and spawner abundance. For example, $\delta^{15}N$ enrichment in coho salmon parr leveled off with increasing spawner abundance in western Washington streams, (Bilby et al. 2001). Such a saturating relationship would limit the ability of $\delta^{15}N$ to resolve among different levels of spawner abundance, particularly at levels above the asymptote. On the Kenai Peninsula, by contrast, $\delta^{15}N$ in coho salmon parr and Dolly Varden continues to increase across a wide range of spawner abundance (Chapter 3 of this thesis), creating potential for resolution across this range. The above-noted saturating relationship (Bilby et al. 2001) is not necessarily characteristic of western Washington streams, as another study from that region found a relationship that appears to be linear (Reichert et al. 2008).

While the most common application of a $\delta^{15}N$-based index of spawner abundance would likely be to track site-specific fluctuations in annual escapement, we tested and validated our $\delta^{15}N$-based index across a geographically dispersed set of streams that varied according to spawner abundance. This space-for-time substitution allowed an expedient proof of concept, but this approach subjected our data to potentially high levels of among-site variability, such as different population densities of stream-dwelling fishes,

different species of spawning salmon, different physiographic conditions, and different traditional spawner survey methods. While the isotopic approach appears somewhat robust to these differences, a more realistic test would focus on specific streams where spawner densities vary over time.

Another question that arises from the space-for-time substitution is the extent to which $\delta^{15}N$ in stream-dwelling fishes actually tracks year-to-year fluctuations in spawner abundance. If substantial amounts of MDN are stored and recycled within freshwater ecosystems for extended periods of time (e.g., in sediments, riparian plants, biota, etc.), the nitrogen pool of these systems could become isotopically enriched in proportion to the long-term abundance of salmon within the system. This effect would be especially likely in Pacific coastal streams where ambient nitrogen levels are typically low. Under this scenario, the $\delta^{15}N$ of stream-dwelling fish could equilibrate to the nitrogen pool and $\delta^{15}N$ fluctuations in response to year-to-year variation in escapement might be dampened. This question would also be resolved by studies that track $\delta^{15}N$ over time at streams with highly variable salmon escapements.

These questions clearly indicate that more work is needed, but our study demonstrated that isotope-based estimates of spawner abundance can do a reasonable job of reflecting estimates derived from traditional monitoring methods. Although the study was carried out on the southern Kenai Peninsula, we expect this technique may be transferable to other areas of Alaska and the Pacific Rim. Given adequate refinement and testing, application of the proposed method will allow managers to track escapement at an unprecedented number of sites with minimal field effort and expense.

In addition to the above management implications of this study, MDN is recognized as an ecologically important flux of biomass to stream ecosystems. They provide food and nutrients to stream and riparian food webs and potentially initiate a positive feedback loop whereby spawning salmon increase the productivity of their natal streams (Levy 1997, Willson et al. 1998, Naiman et al. 2002). While a growing body of research has demonstrated the ecological importance of MDN, relatively little progress has been made toward testing and standardizing methods for measuring MDN assimilation in stream food webs. Refinement and widespread application of an isotope-based escapement index would facilitate long-term and spatially extensive monitoring of MDN assimilation as a means to understand the potential effects of fluctuating salmon abundance and other ecological conditions on the food webs of salmon streams.

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Table 4.1. Salmon spawner biomass derived from traditional spawner survey and census methods at 31 Kenai Peninsula study sites.

Study sites	Salmon biomass (MT/km)			Dominant salmon species
	2004	2005	2006	
Anchor R.		1.2	1.1	Chinook/coho
Anchor R., North Fork (downstream)	0.3			Chinook/coho
Anchor R., North Fork (middle)	0.3	0.4		Chinook/coho
Anchor R., North Fork (upstream)	0.3	0.4		Chinook/coho
Anchor R., South Fork (downstream)	2.0	2.5		Chinook/coho
Anchor R., South Fork (middle)	2.0	2.5		Chinook/coho
Anchor R., South Fork (upstream)	0.7	0.8		Chinook/coho
Barabara Cr. (downstream)	1.1			pink
Barabara Cr. (middle)	0.4			pink
Barabara Cr. (upstream)	0.4			pink
Bear Cr.		8.9	6.7	sockeye
Crooked Cr.		0.6	0.6	Chinook/coho
Glacier Cr.		3.9	4.8	sockeye
Happy Valley Cr. (downstream)	0	0		none
Happy Valley Cr. (middle)	0	0		none
Happy Valley Cr. (upstream)	0	0		none
Humpy Cr. (downstream)	7.6	28.7		pink
Humpy Cr. (upstream)	7.6			pink
Juneau Cr. (downstream)		0		none
Juneau Cr. (middle)		0		none
Juneau Cr. (upstream)	0			none
Moose Cr.		0.2	0.7	sockeye
Ninilchik R.		0.8	0.8	Chinook/coho
Ptarmigan Cr.		1.2	1.4	sockeye
Quartz Cr. (downstream)	2.3	1.7	7.1	sockeye
Quartz Cr. (middle)	0.8			sockeye
Quartz Cr. (upstream)	0.8	0.3		sockeye
Russian R. (downstream)	22.3	12.9		sockeye
Russian R. (upstream)	22.3	12.9		sockeye
Windy Bay Left Cr.		13.7		pink
Windy Bay Right Cr.		18.8		pink

Table 4.2. Species (and sample size) of juvenild salmonids collected for $\delta^{15}N$ analysis at 31 Kenai Peninsula stream sites. DV = Dolly Varden char; CS = coho salmon parr.

Study sites	Sampling event			
	Spring 2005	Fall 2005	Spring 2006	Fall 2006
Anchor R.			CS(5), DV(5)	CS(5), DV(4)
Anchor R., North Fork (downstream)	DV(2)			
Anchor R., North Fork (middle)	DV(3)	DV(1)		
Anchor R., North Fork (upstream)	DV(8)	DV(5)		
Anchor R., South Fork (downstream)	DV(2)	DV(1)		
Anchor R., South Fork (middle)	DV(3)	DV(1)		
Anchor R., South Fork (upstream)	DV(3)	DV(5)		
Barabara Cr. (downstream)	DV(3)			
Barabara Cr. (middle)	DV(3)			
Barabara Cr. (upstream)	DV(3)			
Bear Cr.			CS(5), DV(5)	CS(5), DV(2)
Crooked Cr.			CS(4)	CS(4), DV(4)
Glacier Cr.			CS(4), DV(3)	CS(4), DV(4)
Happy Valley Cr. (downstream)	DV(3)	DV(3)		
Happy Valley Cr. (middle)	DV(6)	DV(5)		
Happy Valley Cr. (upstream)	DV(3)	DV(5)		
Humpy Cr. (downstream)	DV(5)		DV(2)	
Humpy Cr. (upstream)	DV(5)			
Juneau Cr. (downstream)		DV(4)		
Juneau Cr. (middle)		DV(3)		
Juneau Cr. (upstream)	DV(4)			
Moose Cr.			CS(5), DV(4)	CS(2), DV(2)
Ninilchik R.			CS(4)	CS(5)
Ptarmigan Cr.			CS(5), DV(2)	DV(1)
Quartz Cr. (downstream)	DV(5)		CS(4), DV(4)	CS(5), DV(2)
Quartz Cr. (middle)	DV(5)			
Quartz Cr. (upstream)	DV(5)	DV(5)		
Russian R. (downstream)	DV(2)	DV(1)		
Russian R. (upstream)	DV(3)	DV(5)		
Windy Left Cr.			CS(2), DV(3)	
Windy Right Cr.			CS(2), DV(4)	

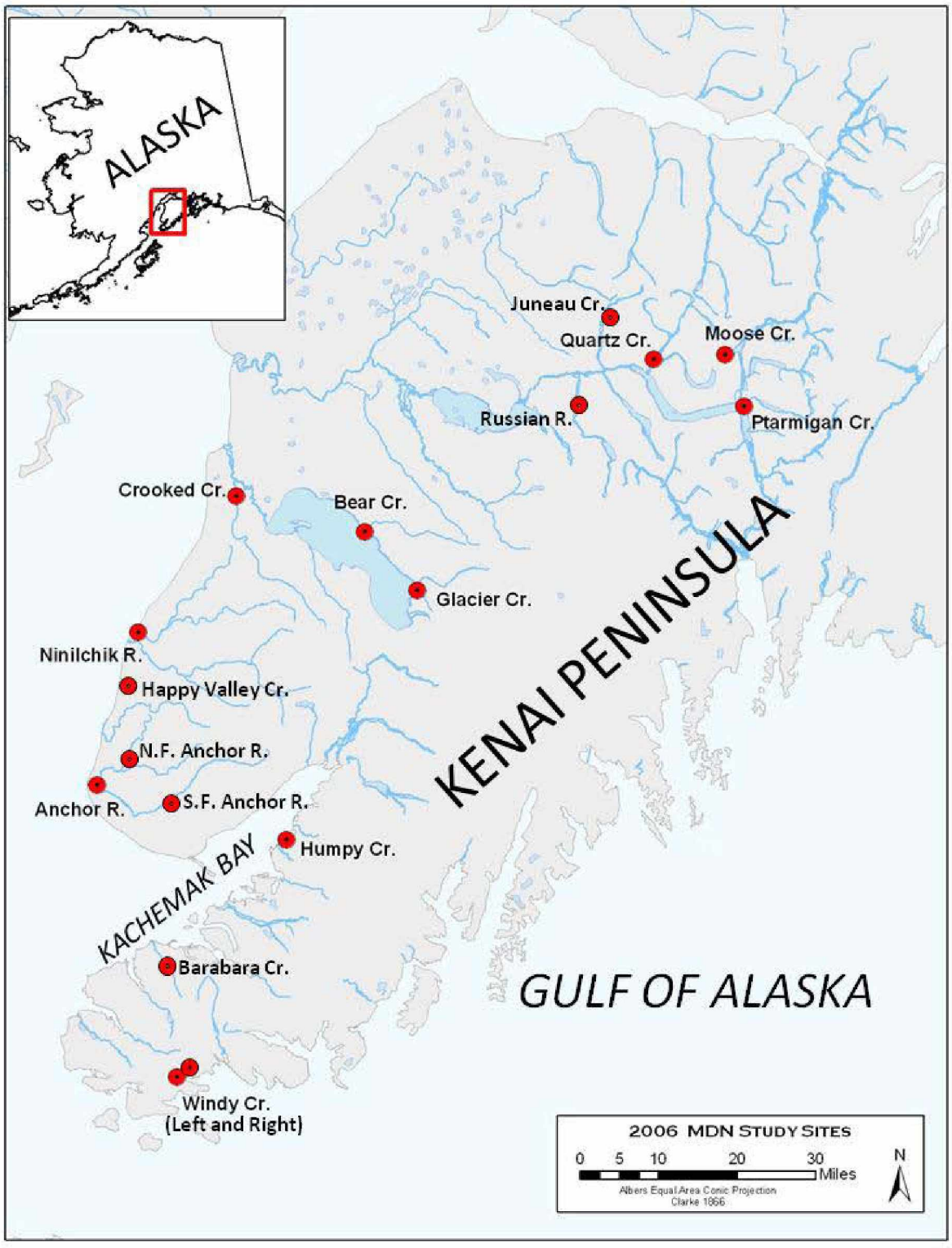

Figure 4.1. The location of study streams on the Kenai Peninsula, Alaska.

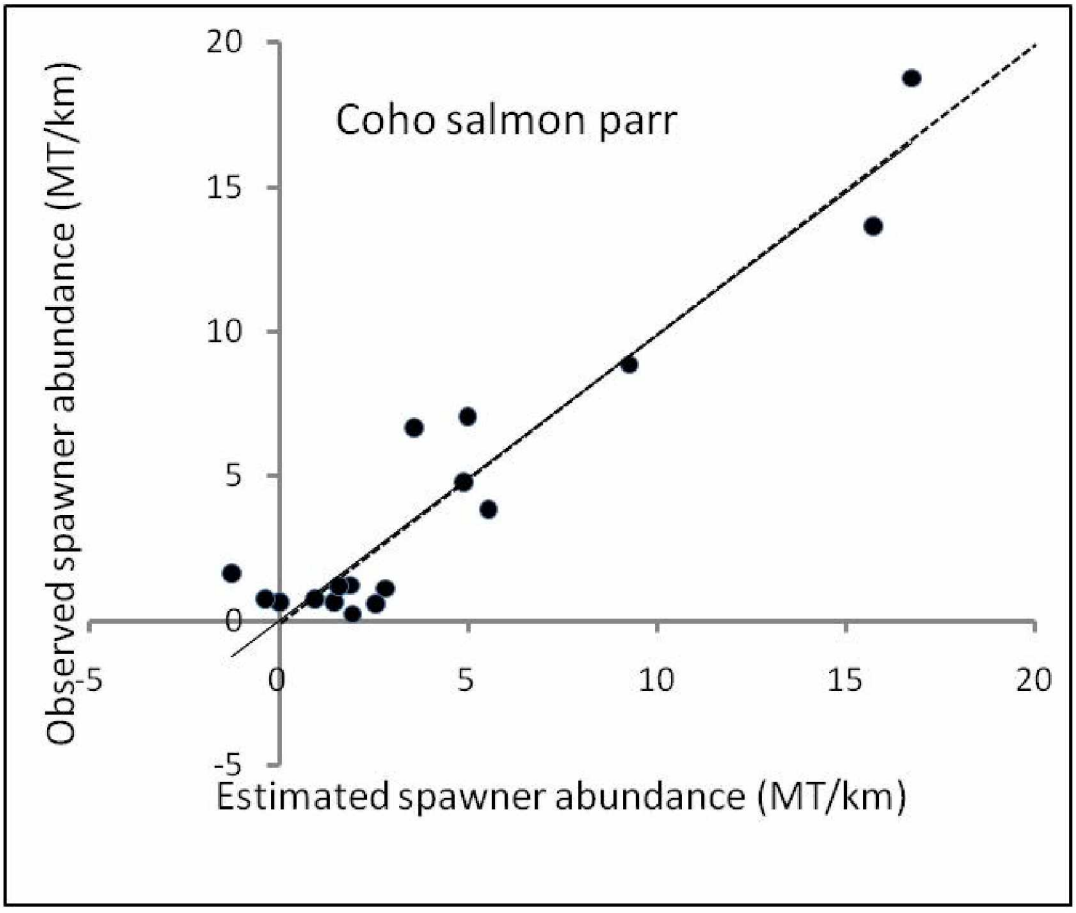

Figure 4.2. Estimated spawner abundance based on nitrogen stable isotopes in coho salmon parr vs. observed spawner abundance. The solid diagonal line is the least squares regression line forced through the origin; the dashed diagonal line represents the 1:1 relationship between estimated and observed.

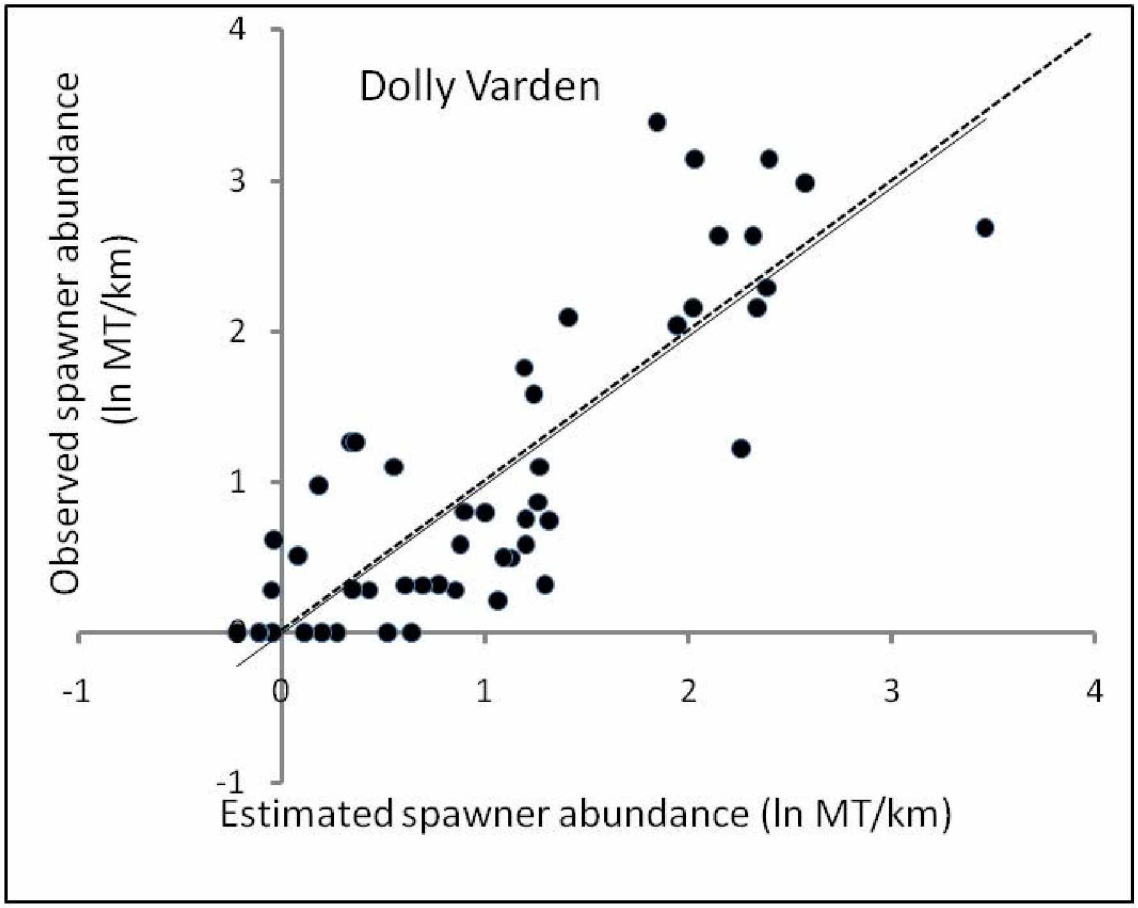

Figure 4.3. Estimated spawner abundance based on nitrogen stable isotopes in juvenile Dolly Varden vs. observed spawner abundance. The solid diagonal line is the least squares regression line forced through the origin; the dashed diagonal line represents the 1:1 relationship between estimated and observed.

Chapter 5

Conclusions

Marine-derived nutrient tracking techniques

MDN are an ecologically important resource flux that contributes to the productivity of salmon spawning ecosystems. The assimilation of MDN by freshwater and riparian biota can be tracked biochemically, and refinement of MDN tracking methods will foster the development of ecologically-based watershed and fisheries management approaches. Chapter 2 sought to understand the factors that influence various stable isotope and fatty acid measures of MDN assimilation in stream and riparian biota and to examine the ability of these measures to differentiate among locations that vary in spawning salmon biomass.

Region was an important predictor for every MDN metric, suggesting that regions have unique backgrounds and that salmon effects are additive upon these background values. Nitrogen isotopes most clearly demonstrate this phenomenon: for all taxa considered in this study, nitrogen isotope values were highest in Homer streams, moderate in Cooper Landing, and lowest in Seldovia at a given spawner abundance (including zero salmon). For all Dolly Varden MDN metrics, fish length was an important predictor, suggesting size-mediated differences in MDN assimilation where larger Dolly Varden consume more MDN than smaller individuals. Season had little or no effect on measures of MDN assimilation in Dolly Varden, which is somewhat remarkable given that samples collected during spring followed the previous spawning

run by ~6 months. Contrary to the results for Dolly Varden, MDN signatures in macroinvertebrates and horsetail tended to vary seasonally, with values higher during summer and/or fall than during spring.

Dolly Varden appear to be better indicators of MDN abundance than the other biota examined in this study. Of the MDN metrics considered, Dolly Varden $\delta^{15}N$ and $\delta^{34}S$ offered the best combination of effect size and precision. We hypothesize that this outcome is related to the high mobility of Dolly Varden which permits them to move as necessary to capitalize on patchily-distributed MDN resources. It is also worth noting that nitrogen isotopes were consistently better predictors than carbon isotopes, explaining up to twice the variation in spawner abundance for all taxa where both isotopes were modeled (i.e., Dolly Varden, shredders, and scrapers).

My results confirmed the assimilation of MDN by multiple trophic levels of stream and riparian ecosystems and indicate the ability of stable isotope and fatty acid metrics to reliably detect MDN assimilation within freshwater and riparian biota. An important outcome of this work is confirmation of the ability to estimate the salmon spawner density in a stream based on the isotopic signatures of juvenile fish rearing there. Region and Dolly Varden length should be taken into account when making inferences about spawner abundance based on MDN measures, especially when collecting data over broad spatial scales or from fish of variable sizes. Season was not a good predictor of any MDN metric, suggesting that MDN signals in Dolly Varden are maintained year-round in south-central coastal Alaskan streams and that the timing of sampling may not be of critical importance.

Marine-derived nutrients and fitness responses

Although the nutritional benefits of MDN on freshwater fishes have been established, the amount of MDN required to maximize the growth and nutritional status of freshwater fishes is unknown. Chapter 3 sought to identify salmon spawner levels above which stream-dwelling Dolly Varden and juvenile coho salmon cease to gain physiological benefits as a direct measure of the capacity of fish populations to utilize MDN. My data clearly show that RNA-DNA ratios and energy density of juvenile coho salmon and Dolly Varden measured in both spring and fall were tightly linked with MDN abundance, suggesting that spawner abundance is an important factor in determining the growth rates, energy storage, and, by extension, survival of fishes rearing in the study streams. My data supported a saturating response in energy density and RNA-DNA ratios, where values increased rapidly with spawner abundance until about 1 kg/m^2 and then leveled off. These results indicate that fish populations have a finite capacity to convert MDN resources into somatic growth or energy stores.

I also tested the relationship between spawner abundance and $\delta^{15}N$ as a potential low-cost proxy for the above fitness measures. While $\delta^{15}N$ was tightly correlated with spawner abundance, the data showed little or no evidence for saturation in $\delta^{15}N$ enrichment with increasing spawner abundance. As such, my data indicate that stable isotopes do not necessarily show the same MDN dose-response patterns as more ecologically relevant metrics like growth or energy storage.

Energy density and RNA-DNA ratios correlated positively with the $\delta^{15}N$ enrichment index for coho salmon samples collected in both spring and fall, yet no such relationships existed for Dolly Varden. Assuming that intraspecific variation in the $\delta^{15}N$ enrichment index was primarily driven by differences in MDN assimilation, the positive correlations between the $\delta^{15}N$ enrichment index and energy density in coho salmon indicate equally strong influence of MDN assimilation on energy storage in both spring and fall. The influence of $\delta^{15}N$ enrichment on coho salmon RNA-DNA ratios, by contrast, varied by season. The magnitude of the response was relatively small in the fall, suggesting a priority for energy storage at that time. In spring, $\delta^{15}N$ enrichment had a relatively large effect on RNA-DNA ratios, suggesting that rapid spring growth was augmented by MDN assimilated during the previous year's spawning run.

In terms of potential management strategies, my data indicated that two independent fitness measures in stream-dwelling fishes, RNA-DNA ratios and energy density, showed saturation responses with spawner abundance, supporting the concept of trophic saturation as a tool to identify spawner levels that account for the needs of biota in stream ecosystems. In terms of MDN effects on the broader freshwater and riparian ecosystem, further research should examine the assimilative capacity and saturation responses of other ecosystem components like primary producers, macroinvertebrates, and riparian plants and animals. Finally, this study indicates that the concept of 'over-escapement' should be viewed in the context of the broader ecosystem. While Ricker stock-recruit models indicate that high escapements reduce per-spawner returns for that

species, high numbers of spawners provide benefits to other freshwater and riparian species.

Stable isotope-based escapement indices

Building on the results of the earlier chapters, Chapter 4 sought to develop and test a quick and inexpensive method for estimating spawning Pacific salmon abundance based on $\delta^{15}N$ in stream-dwelling fishes. Such a method could be adopted in situations where existing techniques render monitoring too expensive or otherwise impractical, potentially allowing managers and researchers to track salmon returns at a much larger number of streams than is currently feasible. My results showed that spawner estimates derived from stable isotopes in stream-dwelling fishes were predictive of those derived by traditional census and survey methods, especially when isotope-based estimates were made using coho salmon parr. The mean absolute deviation of estimates made with coho salmon parr translates to roughly 900 pink salmon or 220 Chinook salmon per stream km while the estimates made with Dolly Varden had nearly twice that error. It should also be noted that some amount of the discrepancy between estimated and observed values stemmed from error in the traditional spawner estimates. The level of error associated with this method may render it unacceptable for comparing among or within stream reaches that receive low densities of spawning salmon, but it may prove adequate for detecting large differences among systems and large year-to-year differences within systems (e.g., many pink and sockeye salmon systems).